

# ANIMAL NUTRITION

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## PREFACE

The purpose of this book is to present the principles of nutrition and their applications in feeding practice. While these applications are made to farm animals primarily, consideration is also given to the broader relationships and significance of the basic principles. Much of the newer knowledge of nutrition, which has markedly improved the health and efficiency of both man and his animals, represents discoveries which have been made with the rat. The same underlying principles have found application in the conservation of wild life, the commercial rearing of fish and game, and even in the control or propagation of insects. Differences in anatomy and physiology and in food supply modify the application of the principles but do not destroy their basic usefulness. These facts are brought out in this book by using experiments with a variety of species as illustrations of the discussions presented.

Nutrition is a chemical process and its principles must be presented in chemical terms. It is assumed that the student has an elementary knowledge of organic chemistry and of physiology. The early chapters of this book provide a review and extension of this knowledge as related to those substances and processes with which nutrition deals. These discussions are limited to the essentials of the language in which the principles of nutrition must be presented.

In the discussion of growth, reproduction, lactation, and other body functions particular attention is given to the nutritive requirements of the various species of farm animals. But the major purpose is to teach how the facts of nutrition are being learned experimentally and how they find application in practice. A consideration of the detailed feeding practices which differ for the various species lies outside the scope of this book; but the discussions here included should provide a background for a better understanding of these practices as presented in the production courses for the various species.

The knowledge of the principles of nutrition and of their application is far from complete. New discoveries are constantly being reported. In adding to our knowledge they inevitably cause some modification in the ideas previously held. Inevitably also, some of the reported discoveries prove to be only partially true. A textbook of nutrition must portray these developments, but it must avoid finality in matters which are in dispute or which require further testing as regards their practical applications. Such has been the aim in preparing this text.

Several considerations have governed the citations to the literature which have been made and the selection of general references included at the end of the various chapters. Some of the classical early contributions have been mentioned, and an attempt has been made to show very briefly the course of the development of the newer knowledge of nutrition by referring to typical studies which have marked its progress. Such a background is helpful for evaluating the significance of current contributions and for forecasting future developments. In discussing the very recent contributions to our knowledge citations are made to the experiments on which they are based in order that these publications may be consulted for more detailed information. In presenting current ideas which are in dispute, either as to fact or as to application, the literature which gives the divergent viewpoints is cited so that the student may have the opportunity to form his own judgment. The general references listed at the end of the different chapters furnish opportunity for collateral reading on the various topics presented. Providing the student with a bibliography of easily accessible sources has been a primary consideration in selecting these various references.

In preparing this text the author has been conscious of his temerity in undertaking the task. He has long felt the need for a textbook for his own classes, and this was the primary reason which led him to attempt the preparation of the present book. The material presented has been selected as a result of some twenty years' experience in teaching the principles of animal nutrition to undergraduate and graduate students at Cornell University. It is hoped that the book may be found useful by other teachers, although it is realized that courses covering the subject matter of animal nutrition are organized very differently at different institutions. The author will highly appreciate

having his attention called to any errors found, and he will be glad to have criticisms and suggestions regarding the nature of the subject matter presented.

Through his long and close association with Professors C. M. McCay, F. B. Morrison, and S. A. Asdell, the author has obtained many ideas which have been incorporated in this book. He gratefully acknowledges his indebtedness to them. He is also indebted to Dr. LeRoy Barnes for assistance in the preparation of the discussion of electromagnetic radiation and of certain other topics of a physical nature. His thanks are due also to Drs. Mary Crowell, G. H. Ellis, George Davis, and E. W. Crampton, and to Russell Rasmussen for reading parts of the manuscript and for many helpful suggestions.

LEONARD A. MAYNARD.

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# ANIMAL NUTRITION

## CHAPTER I

### THE EXPANDING FIELD OF NUTRITION

Today no one needs to be told that tremendous developments are occurring in the field of nutrition. The modern child learns that there are such things as vitamins before he knows his alphabet. In fact, the reader of current popular articles and especially of food advertising is likely to conclude that this field is already overexpanded. At least, he may well wonder why, if all these new ideas in nutrition are so important, his early ancestors survived at all without any knowledge of balanced diets, calories, and irradiated foods. Actually, however, many of our present-day nutrition problems have arisen as a result of the developments which have come with what we call civilization.

With a variety of nature's products at his disposal primitive man found out by trial and error what was safe to eat and what would keep him strong and healthy. This process of self-experimentation unquestionably resulted in many disasters which were repeated as his available food supply changed, but a good diet was always to be found among the plant and animal products available. Certainly the diets of our early ancestors could have been improved by our modern knowledge, but even today those agricultural and maritime peoples who are not prevented by poverty or other limitations from having the varied diet of natural foods selected by their ancestors are in a better state of nutrition than many who are enjoying the so-called improvements of an industrial civilization. For the latter, the character of the basic food supply has been so altered by modern manufacturing, preserving, and cooking processes as to result in troubles previously unknown when the nutrients concerned were always present in the diet.

The milling of cereals which results in a more palatable and more digestible human food, but which removes the better

proteins and most of the minerals and vitamins, is a well-recognized example of this development. Until very recently, the constant trend has been to more refined and less complete foods. The increased consumption of sugar, solely an energy food, to its present level of approximately 120 lb. annually per capita in the United States has displaced more complete foods accordingly and has given rise to some distinctly physiological questions as well. The rise in the use of canned, frozen, and otherwise preserved foods and the decline in food preparation in the home have also created new problems.

The story is similar in animal nutrition. Abraham did not have to worry about a balanced ration for his flocks. Abundant pasture grass from fertile soil provided all that was needed for a successful animal industry. Large performance was not essential in those days. Probably there were no ton litters nor cows producing 10,000 lb. or more of milk yearly. In the course of the centuries and particularly in modern times, many changes have occurred. The development of cultivated crops under an intensive system of agriculture has resulted in a very different and more variable feed supply. Depleted soils have produced forage deficient in certain nutrients, such as calcium and phosphorus. Modern milling practices separate the whole seeds formerly fed into many fractions having different feed values. Various industries are taking more and more of certain products for human food and other uses. This has resulted in a modification of the nature of the by-products available for animals and, frequently, in a lowering of the nutritive value of such products. As these changes have occurred, animals have been constantly developed for greater productive performance. Rapid growth is demanded and higher and higher milk yields are sought. These demands have not only increased the quantity of feed required but have set up many new requirements as to its quality. Thus as the feed supply has become more variable and, frequently, poorer in nutritive value, the needs of the animals have greatly increased and become more exacting. It is not surprising that successful feeding practice is a much more complicated affair than it was years ago and that it requires more scientific knowledge accordingly.

The situation finds an excellent illustration in the poultry industry. Formerly most of the birds ranged the farm, getting

their food from farm and household wastes, grass, worms, and bugs, supplemented by a limited amount of grain. The flocks were small and the production rather low and uncertain, but food was provided for the family table at least. Gradually the industry became an intensive one. Larger flocks were kept, breeding became important and rapid growth, early maturity, and high production were demanded. Confinement in small yards, or even in houses for a part of the year, became necessary. Under these conditions, no amount of the grain formerly fed provided satisfactory nutrition. It was lacking in certain factors which the birds had been able to obtain previously when they were allowed to range the farm, and more of these factors became required to meet the greatly enlarged demands as regards productive performance. Today, in selecting the ingredients of a successful poultry ration, the nature of the protein supplied, the amount of various minerals, several different vitamins, and other specific factors require careful consideration with respect to the purpose for which the ration is to be fed. The modern methods of poultry feeding and management require a real scientific knowledge, but they have developed a much more prosperous industry.

It is these developments which have focused our attention on nutritional requirements previously unrecognized when the system of feeding was such as to supply them fairly well in terms of the limited production expected. The problems presented by these developments have stimulated research which has been responsible for much of our modern knowledge. But the contributions of research have not been limited to the solution of practical problems created by changed conditions. In many instances, under inspired leadership, research has shown the way to better practice before the need for it was recognized. An outstanding example of such a contribution is the feeding experiment with single plants planned by Babcock<sup>1</sup> and carried out at the Wisconsin Experiment Station over 25 years ago.

Babcock, the chemist, recognized that when cows were being fed a combination of several feeds from different plant sources

<sup>1</sup> Stephen M. Babcock (1843-1931), who is most widely known for his invention, the Babcock test, made many pioneer contributions in the fields of dairy chemistry and animal nutrition. Following 6 years at the New York Experiment Station, he served for 25 years as chemist and assistant director at the Wisconsin Experiment Station.

there was no way of knowing what particular contribution each was making to the animals' needs. He conceived the idea of trying out rations made up entirely from a single plant. This proposal seemed decidedly impractical to animal husbandmen who, in those days, thought more of their cows than of the possible value of such an experiment. Eventually Babcock was given the use of two cows, but, when one died after three months, the experiment was abandoned lest another valuable animal be lost also. Later his idea was carried out in an extensive experiment by his younger colleagues, Hart and Humphrey, with the later cooperation of McCollum and Steenbock. Five-month-old heifer calves were selected. Four were placed on a ration made entirely from the wheat plant, four on a similar ration from the oat plant, and four on a ration from the corn plant. Another four received a ration of feeds from all three plants. These rations were made up to be alike as regards their contents of all the organic nutrients then known to be essential, and salt was fed in addition.

In the course of the experiment, striking differences developed between the group on the corn plant and the one on wheat. At the end of a year, similar gains in weight had been made, but the corn-fed animals were much the sleeker and more vigorous. When the animals were bred, remarkable differences occurred in the outcome. Each of the corn-fed group produced a normal calf which developed into a vigorous animal, while all of the calves from the wheat group were either dead at birth or died soon after. The corn-fed group produced three times as much milk in the month following calving as did the other group. The later results continued to be strikingly in favor of the corn plant. Exhaustive chemical studies of the feeds and excreta of the cows, and of the tissues of the calves which died or were killed for autopsy, failed to find any explanation of the results. It was concluded that the wheat plant contained something toxic or, perhaps, that it lacked something supplied by corn. It was years before new discoveries provided the true answer.

This experiment made it clear that there were marked differences in nutritive values which could not be detected by any chemical means available at the time and that the current scientific bases for formulating rations were seriously inadequate. More important, the experiment led to the conviction that simplified diets must be used for the solution of nutrition prob-

lems. It stimulated the use of the purified diet method (Sec. 179) which resulted in the discovery of the first vitamin (Sec. 139) in 1913 and which has been so largely responsible for the newer knowledge of nutrition. The report of this Wisconsin experiment<sup>1</sup> is well worth reading by every student. He should enjoy forming his own opinion, in the light of modern knowledge, as to just what was wrong with the wheat ration. The far-reaching influence of this experiment should convince even the most practical-minded person that no one is wise enough to predict in advance what research is of practical value and what is not. Fundamental facts provide the only adequate bases for meeting the problems created by modifications in the nature of the food supply, by increased production demands, and by a changing agriculture. The changes in food supply will certainly continue, and they may become accelerated.

An important contribution of the recent discoveries in nutrition is that they have explained why certain empirical practices arrived at by the trial-and-error method proved satisfactory and that they have eliminated the disasters of this method by providing generalizations which can be used as a basis for selecting an adequate diet no matter how the food supply may change. Centuries ago, as the history of various peoples shows, individuals and groups here and there learned that certain foods would protect them against scurvy. The disease continued to be a scourge because the number of known effective substances were few, because the information was little disseminated, and because many would not accept what they could not understand. When vitamin C was discovered as the effective dietary constituent, a convincing explanation of previous empirical knowledge was afforded, and a means was provided whereby all foods could be classed as effective or noneffective. During the last five years, this vitamin has been identified chemically, synthesized, and even commercially manufactured. It has taken its proper place along with the previously known essential nutrients. There is no longer any mystery about this vitamin, and scurvy has become entirely preventable.

<sup>1</sup> HART, E. B., E. V. McCOLLUM, H. STEENBOCK, and G. C. HUMPHREY, Physiological effect on growth and reproduction of rations balanced from restricted sources, *Wis. Agr. Expt. Sta. Research Bull.* 17, 1911.

The field of nutrition is expanding because each path which is opened up reveals new ones to be explored. The recent findings that copper, manganese, and zinc play important roles in the body have focused attention on the significance of other "trace elements" in nutrition. What was thought to be a single nutritive factor and called vitamin B when it was discovered has proved to consist of several factors differing as regards their distribution in foods and in their physiological effects. Many believe that the discovery of new vitamins is far from ended. Nutrition is a much more complex affair than conceived by workers of the previous century. We have been primarily concerned about being sure to provide sufficient amounts of newly discovered nutrients in the diet, but we have commenced to learn that for some, at least, there is an upper desirable limit of intake as well as a lower one. Vitamin D is essential to provide for the normal calcification of the bones, but too much causes pathological calcification in the arteries and various tissues. Iodine in the ration of the mother during pregnancy is necessary to prevent the offspring being born with goiter, but feeding additional iodine to farm animals when it is not needed has been shown to be distinctly disadvantageous. Certain diets are lacking in iron for normal blood formation, but excessive intakes interfere with calcium nutrition. Ratios between certain nutrients are important as well as the absolute amounts. Of particular importance from the standpoint of economical production in farm animals is the fact that nutritive balance plays an important role in the over-all usefulness of the ration as a whole. We have come to realize the importance of these things, but we are far from knowing the optimum intakes of the various nutrients, either absolutely or in relation to each other. We have learned that adequate and optimum are not always synonymous in nutrition.

We have learned that errors have been made by too broad generalizations in the use of newly discovered facts. Unsuspected limitations sometimes arise when the practical application is made. Cod-liver oil is a desirable addition to the diet, in many situations, from the standpoint of supplying needed vitamins A and D, but it cannot be fed in any considerable amount to a dairy cow without causing a lowering of the percentage of fat in the milk, nor can it be fed to certain growing herbivora without causing muscle degeneration. For several years spinach has



been widely used as a green vegetable for children to supply needed vitamins, iron, and calcium. It has recently been learned that the iron of spinach is not very available to the body and that the large amount of oxalic acid which is present in this vegetable interferes with calcium metabolism and may thus make spinach a liability rather than an asset in calcium nutrition.

As higher and higher productive performance is sought in our farm animals, we recognize the limitations of our measures of adequate nutrition from the standpoint of productive life. The rearing of breeding stock, milk-producing animals, and laying hens represents an initial cost which, for greatest return, must be spread over a long period of profitable production. The desirability of rapid growth, not only to bring animals into production as soon as possible but also to develop the highest producers has been a dominant idea in current nutrition thought. Recent discoveries in nutrition have resulted in feeding practices which have markedly accelerated the growth rate. At the same time, however, an increasing appreciation has developed that a large number of animals, which are highly promising from the standpoint of growth performance, fail to come into profitable production or have an abnormally short productive life. The large turnover of cows is a serious problem in the dairy business. Along with the factors of inheritance and of disease, the nutritional aspect deserves further study. There is need for a reconsideration of the nutrition of the animal from birth, with profitable productive life rather than rate of growth as the dominant viewpoint.

In a recent thought-provoking article McCay and Crowell<sup>1</sup> have challenged the current view that the most rapid growth as measured by increase in weight and size is the ideal for maximum health and long life, and they have cited evidence obtained with various species in support of their thesis. Clearly, increase in weight and size is a very inadequate measure of the complete development of the organism. It is possible that in stressing this general measure we have neglected factors vital to the correlated and optimum growth of the diverse organs and glands upon which later production and longevity depend. The enhanced growth in weight and size which has resulted from large

<sup>1</sup> McCAY, C. M., and MARY F. CROWELL, Prolonging the life span, *Sci. Monthly*, 39, 405-414, 1934.

intakes of recently discovered dietary essentials may be accentuating the limiting effect of as yet unidentified factors required for complete development and continued function. There may well be no conflict between rapid growth and length of productive life, provided the growth obtained is complete and correlated in all its aspects. But we may have no adequate knowledge at the present time of how to measure this growth and thus may be providing a nutrition which is far from optimum to achieve it.

The nutrition worker who is a true scientist recognizes the limitations of present knowledge. He realizes that findings which have later proved to be inadequate have been responsible for practical recommendations which did not prove effective. He is becoming more conservative accordingly. But overenthusiasm or worse has characterized many of the popular articles in the field of nutrition and some of those who have food products for sale seem to have no inhibitions at all. It is not surprising that the layman is puzzled by what he reads and that reasoning or actual experience may convince him that the field is over-expanded. There has been an overexploitation of present knowledge, but the need for research to fill in the many gaps in this knowledge cannot be denied, and the accomplishments of the recent decades make it evident that further intensive studies will prove highly beneficial to human and animal welfare. There should be a curtailment of premature conclusions and recommendations for practice and an expansion of critical research by competent workers who have the patience to carry through the long time experiments demanded for the complete solution of current problems.

The great French chemist, Lavoisier,<sup>1</sup> is frequently referred to as the founder of the science of nutrition. He established the chemical basis of nutrition in his famous respiration experiments carried out before the French Revolution. His studies led him

<sup>1</sup> Antoine Lavoisier (1743-1794) introduced the balance and thermometer into nutrition studies. He discovered that combustion was an oxidation, and he showed that respiration in the body involved the combination of carbon and hydrogen with oxygen from inspired air and that the quantities of oxygen absorbed and carbon dioxide given off depended on the food intake and the work done. With Laplace, he designed a calorimeter by means of which it was demonstrated that respiration is the essential source of body heat. The science of nutrition was undoubtedly set back many years when Lavoisier's career was ended by the guillotine.

to state: "La vie est une fonction chimique." Thereafter chemistry became an important tool in nutrition studies. Soon it came to be realized, however, that a knowledge of physiological processes was equally essential in order to understand the nutritional requirements for different functions and how they varied in the different species.

With the discovery of the so-called deficiency diseases, pathology also came into the picture, and the importance of distinguishing physiological abnormalities caused by diet from those due to infectious disease came to be emphasized. Abortion in cattle is sometimes caused by dietary deficiencies, but it is much more commonly the result of infection. Neither aspect can be neglected. The nutrition worker must have an appreciation of the points at which pathology touches his primary field of interest. He must also recognize the governing role of inheritance in animal development. As the nutrition field expands, it becomes increasingly evident that there must be a joint attack which utilizes the techniques of several sciences for the solution of many of its problems. The biological sciences are not alone concerned. Physical techniques have played an important role in the identification of vitamins and provide methods for their assay in biological materials. Mathematics has also made significant contributions.

The modern student of nutrition must have an appreciation of the important relations of these basic sciences to the solution of the problems of this expanding field. He must have a real working knowledge of chemistry and physiology particularly, if he is to understand the present-day developments and if he is to apply them most effectively in feeding practice. Such a knowledge will also help him evaluate the significance of new facts as they are discovered and modify his practice accordingly. And practice means human nutrition as well as animal, for everyone at least feeds himself. It is with these view points that the subject of nutrition is treated in this book.

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## CHAPTER II

### THE ANIMAL BODY AND ITS FOOD

Nutrition involves various chemical and physiological activities which transform food elements into body elements. At the outset, therefore, a brief consideration of the chemical composition of the animal body in relation to the composition of its food is useful to give a general picture of the nutrition process, the detailed aspects of which are presented in later chapters.

#### COMPOSITION OF THE ANIMAL BODY

Over 75 years ago the famous English scientists, Lawes and Gilbert<sup>1</sup> performed the pioneer and laborious task of analyzing the entire bodies of farm animals. Since that time, many similar studies have been made by other workers, with the result that we have a large body of data regarding the composition of various species at different ages and in varying states of nutrition. From these data the figures given in Table I have been assembled to provide a general picture of the gross composition of mature animals in a good state of nutrition and to show that different species are very similar in this respect.

**1. Water and Organic Substances.**—The data in Table I, given in round numbers, are subject to large variations according to age and nutritional state, as well as to differences among individuals. The water content shows a large decrease with age in early life. In the case of cattle, for example, the water content is approximately 95 per cent for the embryo shortly after concep-

<sup>1</sup> Agricultural science owes a tremendous debt to John B. Lawes (1814–1900) and Joseph H. Gilbert (1817–1901) for their pioneer work in the fields of agronomy and animal nutrition, begun in 1843 and continued for over half a century. The experiment station which they founded at Rothamsted, England, rapidly gained fame throughout the world, and it remains today an outstanding center of research in the plant sciences. Their studies of the composition of the animal body are published under the title: *Experimental enquiry into the composition of the animals fed and slaughtered as human food*, *Trans. Roy. Soc. (London)* 2, 493–680, 1859.

tion, 75 to 80 per cent at birth, 66 to 72 per cent at five months, and 50 to 60 per cent in the mature animal. The variations for a given age are due primarily to the nutritional state as reflected in the store of fat. Very fat animals have less than 50 per cent of water at maturity. The fat content normally increases with age, but it is highly variable at all times depending upon the level of food intake. Its variation affects the percentages of the other constituents, and this is particularly true for water. The Missouri workers, for example, found a thin steer to contain 18 per cent of fat and 57 per cent of water, in contrast to 41 per cent of fat and 42 per cent of water for a very fat animal.

In view of the wide differences in fat content which may occur, much less variable figures for the other constituents are obtained by expressing them on a fat-free (protoplasmic) basis. On this basis the gross composition of the mature animal body, less the contents of the digestive tract, is represented by the following

TABLE I.—PERCENTAGE GROSS COMPOSITION OF THE ANIMAL BODY<sup>1</sup>

Species	Water	Protein	Fat	Mineral matter
Steer.....	55	17	23	4.6
Hog.....	58	15	24	2.8
Sheep.....	60	16	20	3.4
Hen.....	56	21	19	3.2
Mare.....	60	17	17	4.5
Mouse.....	65	17	13	4.5

<sup>1</sup> Less contents of digestive tract.

approximate figures: water, 75 per cent; protein, 20 per cent; and mineral matter, 5 per cent. These figures are subject to little variation after the animal is nearly full grown, though there is a slight decrease in water throughout life.

The data of Table I do not reveal the very small amount of carbohydrate which is present in the body. Though occurring as much less than 1 per cent at any given moment, it is constantly being formed and broken down in metabolism and thus performs a multitude of vital functions. The only species differences shown in the table which may be considered significant are certain ones for mineral matter, reflecting differences in size of skeleton.

The chemical groups which make up the gross composition of the body are not evenly distributed throughout the various organs and tissues but are more or less localized according to their functions. Water is an essential constituent of every part of the body, but its quantitative distribution varies greatly in different parts. Blood plasma contains 90 to 92 per cent, muscle 72 to 78 per cent, bone approximately 40 per cent, and the enamel of the teeth 5 per cent.

Proteins are present in every cell and, as such, are the principal constituent, other than water, of the organs and soft structures of the body such as the muscles, tendons, and connective tissues. Most of the fat is localized in the adipose tissue, or fat depots, which occur under the skin, around the intestines, around the kidneys, and other organs; but it is also present in the muscles, bones, and elsewhere. In fact every cell contains substances classed with the fats. The small amount of carbohydrate present in the body is found principally in the liver, muscles and blood.

**2. Mineral Composition.**—The mineral matter of the body comprises a large number of elements present in varying amounts in different parts, according to the functions they perform. The percentages of the principal mineral constituents of the body are indicated by the following data:

Element	Per cent	Element	Per cent
Calcium.....	1.33	Chlorine.....	0.11
Phosphorus.....	0.74	Magnesium.....	0.041
Sodium.....	0.16	Sulfur.....	0.15
Potassium.....	0.19	Iron.....	0.013

These data are averages of analyses of eighteen steers of varying ages, as reported by Hogan and Nierman.<sup>1</sup> They are expressed as a percentage of the entire body less the contents of the digestive tract. It is noted that, aside from calcium, the elements occur as fractions of a per cent only. Despite their small amounts, they are absolutely essential to life.

<sup>1</sup> HOGAN, A. G., and J. L. NIERMAN, Studies in animal nutrition. VI. The distribution of the mineral elements in the animal body as influenced by age and condition, *Mo. Agr. Expt. Sta. Research Bull.* 107, 1927.

Calcium, the mineral occurring in largest amounts in the body, is present almost entirely in the bones and teeth as phosphate and carbonate. The phosphorus which is combined with calcium to form the skeleton accounts for approximately 80 per cent of the body supply. The remainder is widely distributed in combination with certain proteins and fats and as inorganic salts. Sulfur occurs throughout the body as a part of the protein molecule. Sodium, potassium, and chlorine are present almost entirely as inorganic salts in the various fluids. Most of the magnesium is present in the bones, but it is also found widely distributed elsewhere in the body. Iron is an essential constituent of the hemoglobin of the blood and occurs in lesser amounts throughout the organs and other tissues.

In addition to the elements listed in the table, there are many others which are present in smaller amounts, some of which are known to be necessary for life. Iodine is an essential constituent of the thyroid gland, and copper, zinc, and manganese are also essential elements which occur in the body in traces only. Boron, silicon, bromine, aluminum, nickel, fluorine, and arsenic are additional elements which have been reported as normally occurring in the body, though they have no known function. Certain other mineral elements have been detected spectroscopically.

**3. The Blood.**—From the standpoint of nutrition, the composition of the blood is of special importance in that it is the medium by which the nutrients are carried to the various parts of the body and by which the waste products of metabolism are removed. The blood makes up from 5 to 10 per cent of the body weight depending upon the species and nutritive state. The figure for birds is higher than for mammals. The blood volume is related primarily to the active tissues of the body. Thus the larger the amount of adipose tissue the lower is the percentage of blood for the body as a whole. For example, it has been shown in the case of the hibernating woodchuck that the figure for blood percentage increases as he uses up his fat reserve.

The corpuscles make up from 30 to 45 per cent of the blood, depending upon the species. This percentage value is called the *hematocrit*. The solid matter of the red corpuscles consists almost entirely of the iron-containing protein, hemoglobin. In certain lower forms, however, the protein of the corpuscles contains an



element other than iron as its respiratory pigment. The *Pinna squamosa*, shell fish, have a protein called pinnaglobulin which contains manganese. Lobsters, crabs, and snails have the copper-containing hemocyanin.

The plasma contains 10 per cent of solids, more than half of which are proteins. The remainder consists principally of various fatty substances, sugar, nonprotein-nitrogen compounds, and inorganic salts. The principal inorganic elements are sodium and chlorine, with potassium, calcium, magnesium, phosphorus, and others occurring in much smaller amounts. Most of the sodium and chlorine are combined together, but various other combinations of these and other elements occur, such as sodium bicarbonate, disodium phosphate, and potassium chloride.

#### COMPOSITION OF PLANTS AND THEIR PRODUCTS

Food must supply nutrients which can be used to build and renew the components of the body and to form its products such as milk, eggs, and wool, and it must furnish energy for the processes involved. After weaning, most of our farm animals obtain all of their food supply from plants. While there are certain animal species which are entirely carnivorous, the plant kingdom is the original and essential source of all animal life, because plants are able to utilize the energy of the sun to build substances which will nourish the animal. Plants make use of carbon dioxide, water, nitrates, and other mineral salts to form carbohydrates, fats, and proteins which the animals must have to build their bodies and which are broken down in life processes. Thus plants store and animals dissipate energy.

**4. Plants and Their Parts.**—Plants contain the same substances that are found in the animal body, but the relative amounts present are very different. Plants also show much larger differences in composition among species than do animals. The composition of certain typical plants and plant products is given in Table II. These data are presented for the purpose of comparing them with the data previously given for the animal body (Table I) and of illustrating certain useful generalizations regarding differences in composition among plants and their various products.

The analyses of three green plants are given to show the general composition of the living plants at the stage when vegetative growth is practically completed but before the seed has matured. These data reveal the fact that the principal constituent of living plants is water even as is true of the animal body. This water content decreases as the seed is matured. The striking difference in the composition of plants and animals is the fact that the dry matter of plants consists principally of carbohydrate.

TABLE II.—PERCENTAGE COMPOSITION OF TYPICAL PLANTS AND THEIR PRODUCTS<sup>1</sup>

Plants	Water	Protein	Fat	Carbohydrates	Mineral matter	Calcium	Phosphorus
Green plants:							
Corn.....	73.1	2.1	0.7	22.8	1.3		
Alfalfa.....	74.1	4.4	0.8	18.2	2.5		
Timothy.....	67.9	2.7	0.9	26.5	2.0		
Dried plant products:							
Corn stover.....	9.4	5.9	1.6	77.3	5.8	0.46	0.09
Corn grain.....	12.8	9.6	3.9	72.3	1.4	0.01	0.28
Soybean.....	9.8	36.9	17.2	30.8	5.3	0.20	0.60
Alfalfa leaves.....	11.0	21.9	3.0	53.9	10.2	2.25	0.23
Alfalfa stems.....	11.4	10.0	1.2	70.8	6.6	0.79	0.14

<sup>1</sup> These data are taken from Appendix Table I in "Feeds and feeding," 20th ed., by F. B. Morrison, by permission of the Morrison Publishing Company, Ithaca, N. Y. The figures for alfalfa and timothy are the averages for plants in bloom, while those for corn represent its composition in the dough-to-glazing stage.

This constituent serves as both structural and reserve material, while in animals protein comprises the structure of the soft tissues and fat is the reserve. Thus, although the animal body contains only a trace of carbohydrate, this nutrient is the principal constituent of its food. It serves principally as a source of energy, either currently, or as a reserve in the form of fat, into which it is readily transformed.

The data for dried plant products, representing the moisture basis to which they are reduced after curing for storage, are given to bring out certain generalizations regarding differences in composition among the various parts of the plant. The figures for corn stover and corn grain provide a comparison between the

vegetative portion of the plant and its seed. The data for the soybean illustrate some characteristics of legume seeds and those for the alfalfa products bring out certain differences between leaves and stems.

Protein is primarily a constituent of active tissues and thus leaves are much richer in this nutrient than are stems, as the data for alfalfa show. Leafy, legume hays such as alfalfa and the clovers always contain more protein than the grass hays such as timothy. As the plant matures, there is a movement of protein from the vegetative parts to the seed to provide for the requirements of growth during germination. Thus, at maturity, the seed contains a higher percentage of protein than the rest of the plant, as is indicated by the figures for corn grain and corn stover.

Fat is also higher in the leaves than in the stems and generally is highest in the seeds where it serves as a condensed reserve of energy for later germination. In most seeds, of which corn and other cereals are examples, the principal store of energy is in the form of carbohydrates, but oil-bearing seeds, such as the soybean, cottonseed, and flax, contain their reserve primarily as fat, as their name implies. These seeds are used as commercial sources of oil, leaving oil meals as by-products for animal feeding. Oil-bearing seeds are also much higher in protein than are the cereal seeds.

In all plant products, with the exception of the oil-bearing seeds, carbohydrate is the principal constituent, even as it is in the plant as a whole. The nature of this carbohydrate differs markedly according to whether it is serving as a reserve or structural element. In seeds, it occurs principally as starch which is the reserve carbohydrate, while, in stems and to a much lesser extent in leaves, a considerable proportion of it is present as cellulose, the principal structural carbohydrate. The outer coats of seeds also contain cellulose as a structural and protective element. Since cellulose and related compounds, classed by the nutrition chemist as *crude fiber* (Sec. 39), are much less digestible than starch, the various parts of plants differ markedly in nutritive value according to their digestibility. Feeds which are high in cellulose and related compounds and thus of low digestibility, such as hay, straw, and silage, are classed as *roughages*. The term, *concentrates*, is used to denote those low in cellulose and

highly digestible. Here are included the seeds and most of their by-products.

**5. Mineral Matter.**—The amount of mineral matter in plants is highly variable in different species as well as in the different plant parts. From the standpoint of animal nutrition, we are particularly interested in the fact that the percentage distribution of the mineral elements of plants differs markedly from that in animals. To illustrate this fact for the dried plant products, data for calcium and phosphorus, the elements which make up over 70 per cent of body ash, are given in Table II. With the exception of the legumes which are always rich in calcium, these elements make up a rather small part of the ash of plants. Both are exceeded by potassium, an element in which we are much less interested in animal nutrition. Calcium is primarily associated with the vegetative portion of the plant, and the leaf is much richer than the stem. Without exception, seeds are low in calcium compared to the other parts of the plant, though oil-bearing seeds are higher than others. In contrast to calcium, phosphorus is much richer in the seeds than in the rest of the plant. Leaves are richer than stems. The calcium and phosphorus content of the vegetative part of the plant is markedly influenced by soil factors. This is fully discussed later (Sec. 111).

TABLE III.—PERCENTAGE DISTRIBUTION OF NUTRIENTS IN THE PARTS OF THE WHEAT KERNEL

Part of wheat kernel	Protein	Fat	Starch, sugar, etc.	Cellulose, pentosans, etc.	Ash	Undetermined
Whole kernel.....	11.3	2.2	66.4	8.0	2.0	10.1
Endosperm.....	11.2	1.2	81.4	2.1	0.4	3.7
Seed coats.....	17.6	8.3	7.0	43.9	8.6	14.6
Embryo.....	40.3	13.5	24.3	1.7	4.8	15.4

**6. By-product Feeds.**—The feeds of animals obtained from plants consist not only of forage crops, seeds, and roots but also of by-products arising from the processing of various plant materials, notably seeds, in the manufacture of products used for human food and for industrial purposes. The bran and middlings which arise from flour milling, gluten feed which is a by-product of cornstarch manufacture, and the meals which are the residues

of the pressing of oil from oil-bearing seeds are all familiar examples of the very large number of by-product feeds. Their composition is usually very different from that of the seed or other material from which they arise. This is illustrated by the figures in Table III which are taken in a condensed form from data presented by Osborne and Mendel.<sup>1</sup> While these data must be considered as approximate only in view of the rather large percentage of undetermined material, they serve to show the large differences in composition among the different parts. The endosperm consists very largely of starch, the reserve material, and contains very little of the less digestible carbohydrates. In contrast, the seed coats are characterized by a high content of cellulose and related compounds which provide the needed protective qualities. They are also richer in protein, fat, and mineral matter than the endosperm, or the seed as a whole. The embryo is especially rich, compared to the other parts, in protein and fat and is lowest of all in cellulose.

These combined by-products contain, on a dry-matter basis, approximately 18 per cent of protein, 5 per cent of fat, 60 per cent of starch, 12 per cent of cellulose and other higher carbohydrates, and 5 per cent of mineral matter. Thus the milling of wheat leaves for animals a feed which is richer in protein, fat, and mineral matter than the entire kernel, but which is somewhat less digestible because of the larger amount of the higher carbohydrates. It is the endosperm which provides the patent flour obtained in the commercial milling of wheat. A yield of approximately 70 per cent is obtained, which means that a portion of the endosperm is left behind with the seed coats and embryo which constitute the by-products of the milling process.

<sup>1</sup> OSBORNE, THOMAS B., and LAFAYETTE B. MENDEL, The nutritive value of the wheat kernel and its milling products, *J. Biol. Chem.*, **37**, 557-601, 1919. Osborne (1859-1929), chemist of Connecticut Agricultural Experiment Station at New Haven, and Mendel (1872-1935), professor of physiological chemistry in Yale University, collaborated in nutrition research for over twenty years. Their outstanding discoveries, particularly in the fields of proteins and vitamins, which are frequently referred to in this book, assure them lasting recognition as pioneers in developing the newer knowledge of nutrition. In addition to their joint work, Osborne became the leading authority of the world on the vegetable proteins, while Mendel made many important contributions on various aspects of nutritional physiology and was an inspiring teacher to a host of students who are now carrying on his work in many laboratories.

While wheat by-products have a high feeding value, this is by no means true for all by-product feeds. Oat mill by-product, for example, which is the residue from oatmeal production, contains less than half as much protein and over twice as much fiber as the seed itself, because it consists mostly of the hull. It is therefore of low digestibility and nutritive value. A knowledge of milling processes from which by-product feeds arise and thus of their make-up in terms of the different parts of the seed is a very helpful guide to their composition and feeding value. This subject is covered very thoroughly in the textbook by Morrison.<sup>1</sup>

<sup>1</sup> F. B. MORRISON, Feeds and feeding, Morrison Publishing Company, Ithaca, N. Y.

## CHAPTER III

### SOME PHYSICOCHEMICAL BASES OF LIFE PROCESSES

While most biological reactions can be duplicated in the test tube, the living organism can perform quickly and easily reactions which in the test tube require prolonged intervals or special conditions, such as high temperature. For example, it takes a 30 per cent solution of hydrochloric acid 5 hr. at a temperature above  $100^{\circ}\text{C}.$  to hydrolyze protein, whereas enzymatic hydrolysis in the digestive tract will accomplish the same result in less than half the time at a temperature of  $37^{\circ}\text{C}.$  and in a nearly neutral medium. The body tissues would be destroyed by the strong reagents and the high temperatures of the test tube. Although much remains to be learned as to how biological reactions take place, it is recognized that they are assisted by various physico-chemical phenomena which play special roles in life processes. An understanding of certain of these phenomena is helpful in explaining various nutrition processes.

**7. Surface Phenomena.**—Each cell is separated from its medium and from other cells by a membrane, and the nucleus within the cell is surrounded by a membrane. At these surface boundaries many of the important reactions, both physical and chemical, occur, such as the formation of cartilage and bone which takes place at the boundary between the cell nucleus and its cytoplasm. The molecules or particles of a liquid have a pronounced attraction for each other. At the surface this cohesive attraction is unbalanced, which means that work must be done on a molecule to take it from a position within the body of the liquid to a position in the surface. Thus there is resident in every unit area of surface a certain amount of potential energy which is known as the surface energy per unit area. The *surface energy* per unit area is equal to what is known as the *surface tension*. Since the potential energy of any system always tends toward a minimum, the surface area tends to become as small as possible, as is exemplified by the spherical form of a drop.

Again, since the surface energy tends to become a minimum, any substances present which will lower the surface energy will tend to become concentrated at the surface and this phenomenon is known as *adsorption*. The adsorbed substance lowers the surface energy and hence the surface tension.

**8. Emulsions.**—An emulsion is a suspension of two immiscible liquids in each other, which are held in a more or less permanent suspension by some substance acting as a film or an emulsifying agent. Oil and water, which are immiscible, can be shaken into an emulsion which is fairly permanent by the use of an appropriate emulsifying agent. Fat exists in milk in the form of an emulsion. Emulsification of two liquids may result in a semisolid state, as exemplified by such products as mayonnaise, lanolin which is wool fat with 25 per cent of water added, and the solid alcohol sold under the name of Sterno. The phenomenon is due to surface-tension relations, for the emulsifying substance is a surface-tension reducing agent. Emulsions can be of two types, water in oil or oil in water. If the agent lowers the surface tension of the water more than that of the oil, the residual greater surface tension of the oil causes it to become the inside phase with the water around it. If the agent lowers the surface tension of the oil more than that of the water, the reverse happens. Soaps are common emulsifying agents. Sodium and potassium soaps form oil-in-water emulsions, while calcium and magnesium soaps form the reverse type. Emulsification results in an increase in surface and thus facilitates surface reactions. The bile salts act as the agents in the emulsification of fat in the intestine, whereby a greater surface is afforded for the action of the fat-digesting enzyme.

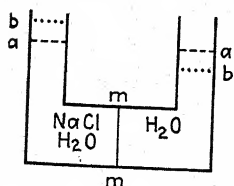


FIG. 1.—Osmotic pressure.

**9. Osmotic Pressure.**—If a solution and the pure solvent are placed in contact a homogeneous solution results under the action of diffusion. If, however, the solution and solvent are separated by a semipermeable membrane, *i.e.*, one through which the solvent can pass but the solute cannot, a special phenomenon arises which is illustrated in Fig. 1. The vessel consists of two compartments separated by a membrane *mm* through which water can pass but dissolved sodium chloride cannot. Water is placed in one com-



partment and a solution of sodium chloride in the other, so that both are at the same level, designated as *a*. Under these conditions, water diffuses through into the salt solution, and the level in this compartment rises while it falls in the other, the final levels being indicated by *b*. This takes place in opposition to the hydrostatic pressure, represented by the differences in level of the two columns, under the influence of a force which is called *osmotic pressure*. This pressure is proportional to the number of particles in solution, whether molecules or ions. Thus, for a given solution, the higher the concentration the greater the osmotic pressure; and, at the same concentration of solute, solutions of highly ionized substances have greater osmotic pressures than solutions of weakly ionized substances.

Since all body fluids contain dissolved substances, they all exhibit osmotic pressure. This pressure is subject to variation according to the kind and concentration of the particles dissolved, and thus osmotic relationships play an important role in physiological processes. If the content of a cell has a lower osmotic pressure than its liquid medium, water tends to pass out of the cell and it shrinks, if the situation is reversed, the cell swells and may burst. For example, when a red corpuscle is placed in a solution of much lower osmotic pressure, it swells and bursts with the liberation of hemoglobin, the red coloring matter of the blood. The phenomenon is called *hemolysis*. Solutions which are alike as regards osmotic pressure are said to be *isotonic*, while the terms, *hypertonic* and *hypotonic*, denote solutions of higher and lower pressure, respectively. A *physiological salt solution* is one containing 0.85 per cent of sodium chloride, a concentration which is isotonic with blood and other tissue fluids. Substances which are injected into the blood or tissues are placed in such a solution so that osmotic relations will not be disturbed.

**10. Donnan Theory of Membrane Equilibrium.**—Where a membrane is permeable to the ions as well as to the solvent, the products of the concentrations of the ions on each side of the membrane must be equal. Biological membranes are of this type, and yet a very unequal distribution of ions on the opposite sides of a membrane is frequently encountered in the body. Donnan has proposed a theory which helps explain this situation. If a solution of NaCl is separated from pure water by a parchment membrane,  $\text{Na}^+$  and  $\text{Cl}^-$  will pass through, and diffusion

explains the final, equal concentrations on both sides. Suppose, however, that the membrane is permeable to  $\text{Na}^+$  and  $\text{Cl}^-$  but not to a given ion,  $\text{R}^-$ , and that at the start we have the following separated by a membrane, (2) being the more concentrated:



$\text{Na}^+$  and  $\text{Cl}^-$  will diffuse from (2) into (1) until at equilibrium the following distribution occurs:



At this equilibrium, the product of the  $\text{Na}^+$  and  $\text{Cl}^-$  on one side of the membrane must equal their product on the other side; but also in (1) the concentration of  $\text{Na}^+$  must equal the concentrations of  $\text{R}^-$  and  $\text{Cl}^-$  to maintain electric neutrality, while in (2) the concentration of  $\text{Na}^+$  must equal the concentration of  $\text{Cl}^-$ . Thus the concentration of  $\text{Na}^+$  is greater in (1), than in (2), and, for  $\text{Cl}^-$ , the reverse is true. In this way it is possible to account for different concentrations of diffusible ions on different sides of a membrane, the essential condition being a nondiffusible ion on one side of a membrane through which all other ions can pass. Many compounds of the body are of the  $\text{NaR}$  type, such as sodium proteinate, and the Donnan theory is very useful in explaining various secretory processes.

**11. The Colloid State of Matter.**—If common salt is dissolved in water, the dissolved particles are of molecular size and cannot be seen even through the ultramicroscope. The solution is clear and no settling occurs. If, on the other hand, fine sand is shaken with water a suspension is formed in which the suspended particles of sand are many, many times larger than the molecules of the dissolved salt, and the particles gradually settle out. While both the dissolving of the salt and the suspension of the sand are purely physical phenomena, the condition of the distributed or dispersed particles and their subsequent behaviour are very different because of the wide difference in their size. An intermediate condition can occur in which the particles dispersed are larger than molecules, but smaller than those of the suspension described. This condition is referred to as the colloid state of matter. The particles are large enough to be seen by the ultra-

microscope, but not large enough to settle out. They are called *colloids* as distinguished from the crystalloids of molecular size in the salt solution. The resulting colloidal solution has many properties which are very different from those exhibited by a true solution such as one of salt and water, and these properties are very important in biology.

The essential characteristic of a colloidal solution is particle size. There is no one size, however, which defines the colloid state. Colloidal solutions may grade into true solutions on the one hand and into suspensions on the other, and the same substance may exist either in a true solution or in the colloidal form depending upon how the solution is prepared. Colloidal properties are exhibited, however, only within a certain range of particle size. The colloidal state of matter has been arbitrarily defined as one in which the size of the particles dispersed lies within the range, 1  $m\mu$  to 100  $m\mu$ .

**12. Properties of Colloids.**—In a colloidal system the distributed particles are spoken of as the *disperse* or *internal phase*, while the solvent is called the *dispersion medium* or *external phase*. Some substances, such as kaolin and various metals, consist of pure solid when in the colloid state and are called *suspensoids* or *lyophobic colloids*, in contrast to certain others called *lyophilic colloids*, because, in aqueous solution, they have an attraction for the water such that the disperse phase may contain large quantities. Most of the colloids which are important in physiology, are of the lyophilic type. Many solutions of lyophilic colloids solidify without the separation of water. This is called *gelling*. The protoplasm of cells of higher animals is a gel and its movements and other activities are due to adsorption and loss of water, actions which are influenced by the salts in the cell fluid and the reaction of the latter. Gelling helps explain permeability. Thus the nutrition of the cell and its aggregates is a function of the above phenomena.

Colloids are only slightly ionized and thus are only slightly reactive chemically. While chemical combinations thus occur, physical combinations are considered by most authorities to be of much more importance. Physical combinations take place through adsorption, a property which is exhibited to a high degree because of the large surface of the colloid particle. Owing to the large size of the particles dispersed and their lack of ionization,

colloid solutions exhibit little osmotic pressure. Colloids diffuse slowly and are unable to pass through a parchment membrane. This property is taken advantage of to separate them from crystalloids in a solution. The process is called *dialysis*.

Most colloids are electrically charged. Placing the colloidal solution in an electric field shows that in some the dispersed particles are electropositive, while in others they are electronegative. The reaction of the dispersing medium plays a role; for example, proteins are positive in acid solution, negative in alkaline solution.

**13. Reaction Velocity, Catalysis.**—The rate of any chemical reaction is proportional to the product of the active masses of the reacting substances. This is the *law of mass action*. The reaction velocity also increases with rise in temperature. More important is the influence of catalysis. A *catalyst* is a substance which alters the speed of a chemical reaction without being used up in the process. Most catalysts accelerate, but negative catalysis is known. In general, the velocity of catalytic reactions is proportional to the amount of catalyst present.

**14. Enzymes.**—An enzyme is a catalyst produced by a living cell. Their activity is an important reason why living tissues can perform reactions much more readily than can be done in the test tube. The roles which enzymes play in the body in digestion and in other processes are familiar to all. Little was known about their specific nature until Sumner isolated in a crystalline state the enzyme, urease (Fig. 2), in 1926, and established its protein nature. Since that time at least five others have been isolated.

One important characteristic of enzymes is their *power*. For example, the enzyme catalase will break down 13,000 times its weight of hydrogen peroxide per minute. Another important property is their *specificity*. A given enzyme can catalyze one particular reaction only. They are characterized also by being effective only in a narrow pH range (Sec. 16). For example, the optimum pH range for pepsin is 1.8 to 2.0 and the outside limits of its activity are pH 1 and pH 3.

**15. Hydrogen-ion Concentration.**—Since most of the reactions which occur in the body take place in water solutions, the ions of water have a special significance in physiology. It is well understood that the acidity or basicity of a solution is due to hydrogen

or hydroxyl ions. According to the theory of Arrhenius<sup>1</sup> their presence and activity are due to the ionization of compounds containing them, and the greater the degree of ionization the greater is the activity, as shown by conductivity measurements. The purest water that can be prepared shows a slight but definite

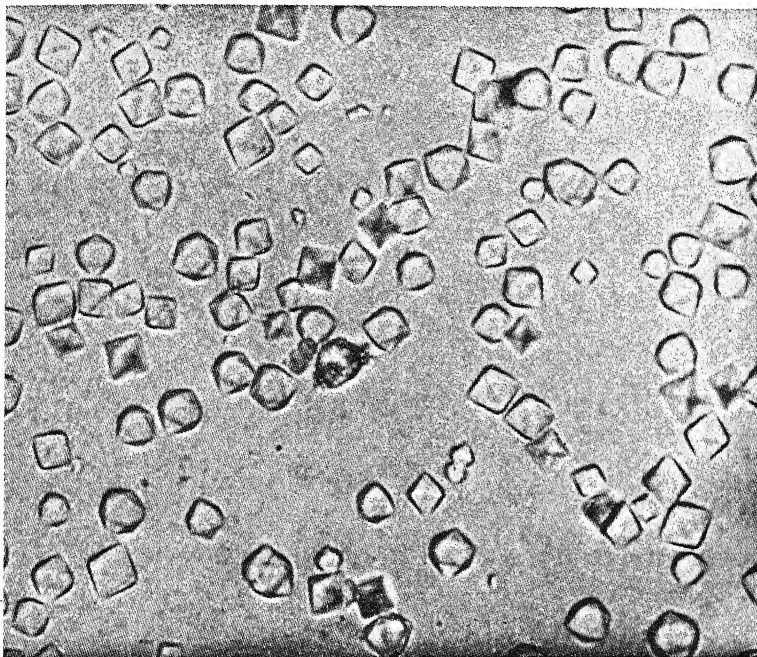
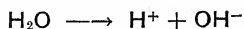


FIG. 2.—Crystalline urease. (Courtesy of J. B. Sumner, Cornell University.)

conductivity. According to the theory, this means that there is some dissociation:



It is possible through conductivity measurements to calculate the number of ions actually dissociated. It has been found that the

<sup>1</sup>Svante Arrhenius (1859–1927) was a Swedish scientist, one of the founders of the modern physical chemistry. Recent research has caused physical chemists to modify his conception in so far as degree of dissociation is concerned and to explain differences in conductivity on other grounds. They speak of activity instead of dissociation and of hydrogen-ion activity instead of hydrogen-ion concentration. This modification does not interfere with the application of the hydrogen-ion concept in physiology.

concentration of each ion, expressed in mols per liter is 1 in 10,000,000 or  $1 \times 10^{-7}$ , an expression which is more easily visualized by writing it as a fraction,  $\frac{1}{10^7}$ . It is also known that the concentration of hydrogen ions  $[H^+]$ <sup>1</sup> times the concentration of hydroxyl ions  $[OH^-]$ <sup>1</sup> equals a constant  $K$ , which is  $10^{-14}$  at 25°C. At neutrality  $[H^+]$  is  $10^{-7}$  and  $[OH^-]$  is  $10^{-7}$ . According to the law of mass action, if the concentration of either ion is increased the other must diminish correspondingly:

$[H^+]$	$[OH^-]$
$10^{-7}$ times	$10^{-7}$ equals $10^{-14}$ (neutral)
$10^{-8}$ times	$10^{-6}$ equals $10^{-14}$ (alkaline)
$10^{-6}$ times	$10^{-8}$ equals $10^{-14}$ (acid)

Thus the measurement of the concentration of one ion will tell us whether the solution is acid or alkaline and the degree. This is what is done where reaction is expressed as the  $[H^+]$  and the solution is acid or alkaline according as  $[H^+]$  is greater or less than  $10^{-7}$ . Concentrations lying between those represented by integral powers of 10 are expressed by coefficients, for example the  $[H^+]$  of saliva is approximately  $2 \times 10^{-8}$  (slightly alkaline).

**16. pH Values.**—Fortunately, we have a simpler method of expressing  $[H^+]$  than the one just described, thanks to the suggestion of Sorenson that the negative exponent be used directly. The value so used is called the *hydrogen-ion exponent* or *pH value*. It bears the following relation to  $[H^+]$ :

$$pH = \log \frac{1}{[H^+]}$$

A pH of 7 represents neutrality, a value below 7 denotes an acid solution and one above 7, an alkaline solution. Decimals take the place of the coefficients used in the expression of  $[H^+]$ . For example, a pH value of 5.6 denotes an acidity greater than 6 but less than 5.

It is essential to remember that the pH scale is logarithmic. A solution with a pH of 6 has 10 times as many hydrogen ions as one with a pH of 7, and a pH of 5 represents a concentration which is 100 times that of pH 7. The difference between pH 5.0 and 5.1 is many times greater than that between 5.9 and 6.0.

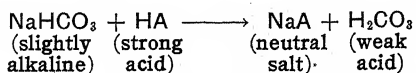
Expressing acidity and alkalinity as a pH value differentiates between strong and weak acids and bases. A  $N/10$  hydrochloric

<sup>1</sup> The brackets indicate "concentration" of the ion whose symbol is enclosed in them.

acid and  $N/10$  acetic acid solution have the same strength in terms of *titrable acidity*. But hydrochloric acid is a strong and acetic a weak acid. Though they both show the same number of hydrogen ions on titration, hydrochloric acid has many more dissociated or active at any moment. pH measures this *actual acidity* as distinguished from the titrable acidity. A  $N/10$  solution of hydrochloric acid has a pH value of 1.09, while a  $N/10$  solution of acetic acid has a value of 2.85 which indicates a much weaker acid in terms of hydrogen-ion concentration. The reactions of the biological organism to acids and bases are controlled by the actual acidity and thus a measure of the hydrogen-ion concentration is more useful than titrable acidity.

The determination of hydrogen-ion concentration can be carried out colorimetrically by the use of standard solutions and indicators which change color at different concentrations of the ion. It is also made directly by the use of a hydrogen electrode. These methods are described in textbooks of physical and physiological chemistry.

**17. Buffer Action.**—The pH of the blood varies between 7.35 and 7.43. The range 7.0 to 8.0 is the extreme compatible with life. Yet the blood constantly receives large amounts of acid, such as  $H_2SO_4$  and  $H_3PO_4$ , from protein breakdown. In order to hold its pH constant under these conditions, the blood contains substances called *buffers* the action of which may be illustrated as follows:



The buffer,  $NaHCO_3$ , reacts with the strong acid to produce an acid of low hydrogen-ion concentration. Buffers are substances which prevent sudden or great changes in hydrogen-ion concentration when acid or alkali is added to a system. All biological reactions take place in a buffered medium because sudden or large changes in acidity or alkalinity are incompatible with life.

**18. Electromagnetic Radiation.**—When one turns on his radio, he is not likely to think of the similarity between the process of sending and receiving the program which he selects and the process of turning on an electric light whereby its filament becomes luminous. Yet both processes involve the transfer of energy through space in the form of waves, and the mechanism is



the same in both cases. The physicist classes both as electromagnetic radiation. In the same class belong the light rays which are responsible for photosynthesis, the ultraviolet rays which produce vitamin D in our bodies and in our foods, the X rays which have manifold uses in physiology and medicine, and many others. Thus the student of nutrition must know something about the nature of this electromagnetic radiation and about the language which the physicist uses in describing it.

When energy is being liberated from the radio sending station, periodic electrical and magnetic disturbances are set up in space surrounding the station and travel away as waves at a velocity which is slightly less than  $3 \times 10^{10}$  cm. per second. It is believed that all the other rays previously mentioned are identical in nature with radio waves and that they travel with the same velocity. All differ, however, in wave lengths and in the frequency of the vibration associated with the wave motion. What is meant by wave length can be pictured by thinking of water waves and the distance from the crest of one to the crest of the next. Velocity, wave length, and frequency bear the following simple relation to each other:

$$c = \nu\lambda$$

in which  $c$  is the velocity,  $\nu$  (nu) the frequency, and  $\lambda$  (lambda) the wave length. Since the velocity is constant for all forms of radiation, frequency and wave length must vary inversely with each other. The relationship is like that between the steps of a boy and a man walking together. The boy takes shorter steps and thus more per unit of time to keep pace with the man.

**19. The Spectrum.**—The physicist has studied electromagnetic radiation ranging in wave length from about  $10^5$  down to  $10^{-5}$  cm. This range is conventionally divided into several loosely defined divisions according to the methods of production and of studying the radiation, giving what is called the electromagnetic spectrum illustrated in Fig. 3. The conventional divisions are given at the top and the wave lengths at various points are indicated at the bottom. Since the differences in wave length are so great from one end of the spectrum to the other, it is convenient to use more than one unit in expressing the wave lengths over the entire range. For the shortest, such as the gamma and X rays, the angstrom unit ( $\text{\AA}$ .) is commonly employed. As the waves



become longer the millimicron or millimu ( $m\mu$ ) comes into use, and, for those which are still longer, the  $\mu$  or micron and finally the centimeter and meter are employed. The rays which are commonly met in nutrition studies are expressed in either angstrom units or millimu. The lengths represented by these units are as follows:

$$\text{\AA} = 0.1 m\mu = 0.0001\mu = 0.00000001 \text{ cm. } (10^{-8} \text{ cm.})$$

It is noted that visible light occupies a very small part of the electromagnetic spectrum. It comprises the wave lengths lying between approximately  $4000\text{\AA}$ . ( $400 m\mu$ ) and  $8000\text{\AA}$ . ( $800 m\mu$ )

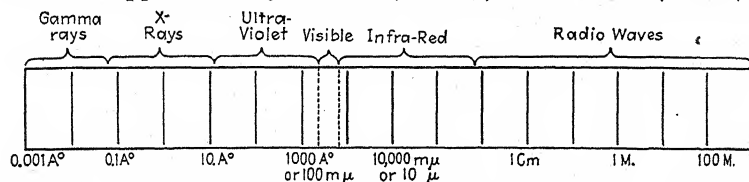


FIG. 3.—Spectrum of electromagnetic radiation. (Courtesy of L. L. Barnes, Cornell University.)

of which the shortest are the violet and the longest the red rays. In addition to visible light, the ultraviolet and X rays are the ones in which we are most interested from the standpoint of nutritional physiology. The infrared waves are also called heat waves because they exhibit a heating effect which can be detected by placing a blackened thermometer in their path. This effect is taken advantage of in the treatment of certain diseases. All of these various radiations represented in the spectrum are commonly included under the term light, though the human eye is sensitive to only a narrow range.

No single source of electromagnetic radiation will provide rays of all lengths. The radiation which reaches us from the sun lies principally in the visible range and in certain portions of the ultraviolet and infrared. Various artificial sources are used to produce wave lengths lying in specific ranges. Ultraviolet rays are commonly obtained from the carbon arc or a mercury-vapor lamp. X rays result when high-speed electrons (cathode rays) strike a solid metal target. Radio waves are produced by vacuum tube oscillators and gamma rays by the spontaneous or artificial disintegration of atomic nuclei.

**20. Absorption of Radiation.**—In considering the effect of radiation on biological processes, it is important to bear in mind

that it is only those wave lengths, absorbed by the medium, which produce any change in it. For example, it is the light which is absorbed by water, not that which passes through, which raises the temperature. Since matter is made up of atoms which consist of electrical charges, it is not difficult to understand that radiation resulting from electrical and magnetic disturbances may in turn cause disturbances in the atoms of matter on which it falls. When radiant energy is absorbed by matter, various processes may occur, such as a rise in temperature, the removal of

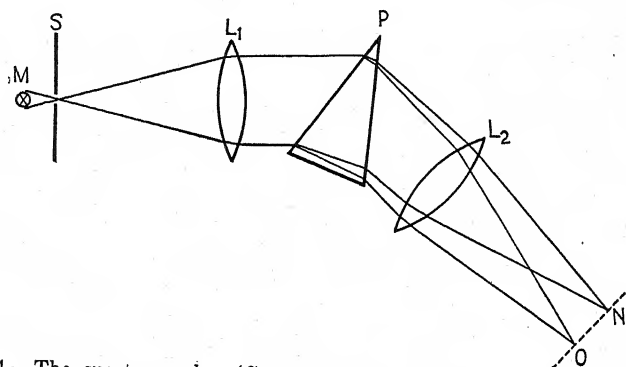


FIG. 4.—The spectrograph. (Courtesy of L. L. Barnes, Cornell University.)

an electron from an atom and various other changes in the structure of the molecules of the substance. These processes involve the transfer of energy from the radiation to the absorbing substance. A given change in the absorbing substance may require a definite amount of energy and this fact determines what wave lengths are effective in bringing about a given change and thus what ones are absorbed in the process. The formation of vitamin D from ergosterol (Sec. 152) by ultraviolet light is an example of a molecular change resulting from the absorption of radiation.

A substance may absorb radiant energy over a wide range of wave lengths, or it may absorb only a very narrow band of radiation. The wave lengths absorbed by a given substance can be determined by a spectrograph such as is illustrated diagrammatically in Fig. 4. A source of light is employed which emits a continuous spectrum, *i.e.*, a source which emits light of all possible wave lengths over a certain selected range, such as  $300\text{ m}\mu$  to  $800\text{ m}\mu$ . When such a source *M* is placed in front of the slit *S* of a spectrograph, the lens *L*<sub>1</sub> makes its rays parallel.

The prism  $P$  refracts the rays, the shorter wave lengths being changed in direction more than the longer ones. As these refracted rays pass through lens  $L_2$ , there will result a broad band of light between  $N$  and  $O$ , the wave lengths becoming shorter and shorter from  $N$  to  $O$ . If now we introduce some substance into the path of the light between  $M$  and  $S$  and if this substance absorbs light of some wave length intermediate between that falling at  $N$  and that falling at  $O$ , then there will be a region between  $N$  and  $O$  which is not illuminated so strongly as it was before the substance was introduced. If a photographic plate is placed in the position  $NO$  the characteristic absorption spectrum of the substance is recorded on the plate. If absorption spectra for light of wave lengths lying in the visible range are being studied, glass lenses and a glass prism may be used, but, for work in the ultraviolet, too much of the radiation is absorbed by the glass itself, and thus quartz must be substituted.

Absorption spectra have recently proven very useful in identifying complex organic compounds and in studying their structure. As is discussed in Chap. VIII, they have contributed largely to our knowledge of the chemical nature of certain vitamins and have also provided methods for their assay.

#### THE ROLE OF WATER IN THE ANIMAL BODY

It has been mentioned that water makes up over 50 per cent of the composition of the body and that most tissues contain 70 to 90 per cent of this substance. In fact one may consider the living elements of the body as water inhabitants even as are the true aquatic species. This water is not simply an inert material or merely a solvent but is an active and structural constituent. If a frog's egg weighing a few milligrams is placed in sterile filtered water, a tadpole weighing several grams results. The tadpole contains less dry matter than the original egg, for a part of it has been used to furnish energy for the developmental process. The increase in weight is due to the taking up of water which has become an essential part of the organism. The vital role of water in the body is indicated by Rubner's<sup>1</sup> observation that the body can lose practically all of its fat and over half of its

<sup>1</sup> Max Rubner (1854-1932), a pupil of Voit, served for over 40 years at the University of Berlin, first as professor of hygiene and later of physiology. He made many pioneer contributions to the science of nutrition, particularly in the field of energy metabolism, as later discussions show.

protein and yet live, while a loss of one-tenth of its water results in death. Adolph<sup>1</sup> in an excellent review of water metabolism has pointed out that water ranks far above every other substance in the body as regards rate of turnover, whether the comparison is made absolutely in gram molecules per day or relatively in per cent of the body content. Its consideration provides an appropriate introduction to nutritional physiology.

**21. Properties and Functions of Water.**—Water is the ideal dispersing medium because of its solvent and ionizing powers which facilitate cell reactions, and because of its high specific heat which enables it to absorb the heat of these reactions with a minimum rise in temperature. Cannon<sup>2</sup> has pointed out that: "The heat produced in maximal muscular effort continued for 20 minutes would be so great that if it were not promptly dissipated it would cause albuminous substances of the body to become stiff like a hard boiled egg." The latent heat of vaporization of water also plays an important role in regulating body temperature. Other properties of large significance in physiology are the high surface tension, the tendency to form hydrates, and the high dielectric constant of water.

The manifold functions of water in connection with the transportation of metabolic products, with secretion and excretion, and with many other body processes are obvious. It plays many special roles also. As synovial fluid, it lubricates the joints, and, as cerebrospinal fluid, it acts as a water cushion for the nervous system. In the ear, it transports sounds, and, in the eye, it is concerned with sight.

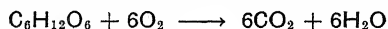
**22. "Free" and "Bound" Water.**—Many physiologists believe that a portion of the water in living material is "bound" to the tissues as distinguished from the familiar "free" water. It is considered that the "bound" water is absorbed by the colloids, that it is a part of the disperse phase as distinguished from the "free" water of the dispersing medium, that it cannot be pressed out, and that it has no solvent power. These properties affect osmotic and other physical relations. In 1922 Rubner noted

<sup>1</sup> ADOLPH, EDWARD F., The metabolism and distribution of water in body and tissues, *Physiol. Rev.*, **13**, 336-371, 1933.

<sup>2</sup> Walter B. Cannon is professor of physiology at Harvard Medical School. The quotation is from his book: "The Wisdom of the Body," W. W. Norton & Company, Inc., New York, 1932.

that a portion of the water present in tissues did not freeze when the tissues were cooled to  $-20^{\circ}\text{C}.$ , and he suggested that this water which does not freeze at this temperature be defined as "bound." There is much experimental evidence for the view that free-bound water relationships play an important role in physiological processes. The winter hardiness of certain wheats and the drought resistant properties of certain plants have been explained on the basis of a high content of "bound" water. Considerable evidence has also been adduced that "bound" water has an important role in the life cycle of certain insects. On the other hand, several physiologists have reported data which fail to support the concept of free and bound water and conclude that only a very small fraction of the water in the body can be associated with the colloids in a "bound" form. It can be expected that further studies will clarify the importance of this highly interesting concept.

**23. Metabolic Water.**—Most of the water which is utilized by the animal body is ingested, either as such or as a component of the food. There is a further available source which is provided by metabolic processes and which is thus called metabolic water. When the carbohydrate, glucose, is burned to furnish energy for body processes carbon dioxide and water result:



By calculations from this equation, it can be shown that the metabolism of glucose yields 60 per cent of its weight as water. This figure can be taken as an approximate one for all carbohydrates. Similarly, the metabolism of protein produces approximately 42 per cent of its weight as water, while in the case of fat the figure is over 100 per cent. Metabolic water is also produced by the dehydration synthesis of body proteins, fats, and carbohydrates. Under certain physiological conditions, metabolic water plays an important role in the animal economy. It suffices to meet the needs of hibernating animals. These animals metabolize their reserves of carbohydrate and fat to provide energy for their vital processes, and this metabolism produces enough water to balance that lost by respiration and evaporation. The various roles of metabolic water in the vital processes of plants and animals have been discussed in a very interesting way by Babcock.<sup>1</sup>

<sup>1</sup> BABCOCK, S. M., Metabolic water: its production and role in vital phenomena, *Wis. Agr. Expt. Sta. Research Bull.* 22, 1912.

#### 24. Factors Governing Water Excretion and Requirement.—

The body's need for water is governed by many factors. Approximately 75 per cent of the tissue formed during growth is water, and, in the mature animal, such special processes as milk and egg production require water in accordance with the amount of the product being formed. Aside from these needs for the formation of tissue and products, there are large requirements for water to balance that lost by excretion through the gut, kidneys, lungs, and skin. These losses are related to body size, and, in rats, a voluntary intake of 800 cc. daily per square meter of body surface has been noted. But the losses are conditioned by body processes and are thus highly variable according to the diet, nature of the metabolic end products, and other factors. The losses through the gut vary with the species, as is evident from the fact that the feces of sheep are drier than those of cattle. A laxative diet increases the fecal loss. Water is excreted in the urine as a solvent for catabolic products which leave the body through this channel, *viz.*, minerals and nitrogenous end products such as urea. The higher the proportions of minerals and protein in the diet, the larger is the excretion of water in the urine and the larger the requirement accordingly. Another reason why a high protein diet increases the water requirement is that less metabolic water is formed in its catabolism than is the case for fat or carbohydrate.

There are marked species differences in water excretion according to the nature of the nitrogenous end products. In mammals the principal end product of protein catabolism is urea which is soluble in water and toxic to the tissues in concentrated solution. Thus much water is required to dilute it to a harmless concentration, remove it from the tissues, and excrete it. Uric acid, the principal nitrogenous end product in birds, is excreted in a nearly solid form with a minimum loss of water. Further, the breakdown of protein to uric acid provides more metabolic water than does its catabolism to urea. Thus, other conditions being equal, birds have a lower water requirement than mammals and are much less sensitive to the temporary deprivation of it. Mammals will live longer without food than without water, and the consumption of food, especially protein food, without water hastens death as the result of the accumulation of toxic end products. Birds, snakes, and insects survive much longer under these conditions.

Clothes moths, which contain 50 per cent of water in their bodies, live throughout their cycle on food containing 10 per cent or less of this compound. They excrete uric acid, and thus the small amount of water obtained as a component of their food, plus their metabolic water, suffices.

Expired air always contains more water than inspired air, and this loss is greatly increased by physical activity and other factors which speed up pulmonary exchange. Perspiration losses through the sweat glands represent an evaporation of water for the dissipation of heat in the regulation of body temperature (Sec. 204). They increase with muscle activity and temperature. In most animals the sweat glands are few or absent. Under these conditions, the lungs play an important role in the dissipation of heat. The constantly occurring excretion of water vapor through the lungs and skin is largely responsible for the body losses referred to as *insensible perspiration* (Sec. 210) which represents the difference between the gaseous intake and outgo. These losses are of considerable magnitude and are related to the environmental temperature and humidity and to the nature of the metabolism including water intake.

Babcock cites the ability of the camel to accomplish long journeys with little water as illustrating the application of various factors which economize water requirement. This animal eats mostly carbohydrate food under these conditions and thus produces little urea for excretion. It depends upon fat stored in its hump for a part of its energy requirement, and the metabolism of this fat in turn provides a maximum amount of metabolic water. Evaporation from the skin is reduced by a thick coat of hair, and the feces are dry. These factors appear to be more important than the water holding capacity of its stomach, the popular explanation. Fasting greatly decreases the need for water. Dogs have been found to ingest only one-fourth as much water on days when no food was given compared to the amount taken on an adequate diet.

**25. Water Requirements.**—It is evident that the body must receive sufficient water to balance its losses in addition to the amount required for the formation of new tissue or products; but it is also clear that the requirement will vary widely according to the magnitude of the various factors which govern the losses, and that there are marked species variations as well. Thus the

determination of the water requirements for a given species and set of conditions is of limited value for any general recommendations. Adolph<sup>1</sup> has devised an elaborate method for estimating water requirements in man. On the basis of this method, he has suggested an intake of 1 cc. per Calorie of ingested food as an approximate figure for the requirement.

Fortunately, except under pathological conditions, there are no deleterious effects from an excessive consumption of water. Thus the requirements can best be taken care of in practice by making sure that the animals have the opportunity to consume all they desire at frequent intervals. The importance of frequent access to water for animals having a high requirement has been clearly shown in studies with milking cows. These animals need 4 to 5 lb. of water for each pound of milk produced. They will consume more when watered twice a day than once a day, and still more if water is before them at all times, and they will produce more milk where they have the latter, free access to water.

**26. The Determination of Water.**—The water present in a biological material is commonly determined by drying it to constant weight at the temperature of boiling water, and this is a satisfactory procedure for most routine analyses. Not all the water, however, at least in certain materials, is removed by this procedure. The unremoved portion represents water existing in films which has a very low vapor pressure even at 100°C. Thus a refined method involves drying in partial vacuum. Strictly speaking, no figures for moisture content can be considered as absolute values since their magnitude is influenced by the three variables involved in the determination—temperature, pressure, and time. When the dried material is to be used for subsequent determinations with which previous oxidation might interfere, the drying is carried out in an inert gas. Certain materials must be dried at a temperature below 100° to avoid alterations in some of their constituents. Other special procedures are required for certain products.

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<sup>1</sup> ADOLPH, *loc. cit.*

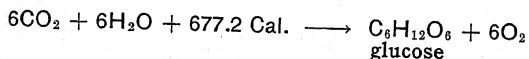


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## CHAPTER IV

### THE CARBOHYDRATES AND THEIR METABOLISM

The group of foodstuffs called carbohydrates includes the sugars, starch, cellulose, gums, and related substances. Though none of these substances, with the exception of a small amount of sugar and glycogen, occur as a constituent of the animal body, they form the largest part of its food. This follows from the fact that carbohydrates make up three-fourths of the dry weight of the plant world upon which animal life primarily depends for its food supply. The carbohydrates in the plants arise by means of photosynthesis, the most important chemical reaction in nature. In this process the plant, with the aid of its chlorophyll, utilizes the energy of the sun to synthesize carbohydrate from carbon dioxide and water. In this way both structural material and a source of energy are obtained for its growth and development. The reaction involves the formation of intermediate products, but it may be simply represented as follows:



The carbohydrates of the plant are in turn used by the animal as a source of energy for its life processes, and, thus, all animal life also is dependent upon the process of photosynthesis.

**27. Classification of Carbohydrates.**—The carbohydrates owe their name to the fact that they contain carbon, combined with hydrogen and oxygen which are usually in the same ratio as in water. Chemically they are polyhydroxy aldehydes and ketones, or substances which yield them on hydrolysis. An abbreviated classification, which includes the members in which we are particularly interested in nutrition, is presented in Table IV.

It is noted that the various members of a given subgroup have the same empirical formula. They have, however, different structural formulas and exhibit different degrees of optical activity. This ability to rotate the plane of polarized light is an

TABLE IV.—CLASSIFICATION OF CARBOHYDRATES

I. Monosaccharides	III. Trisaccharides, $C_{18}H_{32}O_{16}$
1. Pentoses, $C_5H_{10}O_5$	Raffinose
Arabinose	IV. Polysaccharides
Xylose	1. Pentosans, $(C_5H_8O_4)_x$
Ribose	Arabian
2. Hexoses, $C_6H_{12}O_6$	Xylan
Glucose	2. Hexosans, $(C_6H_{10}O_5)_x$
Fructose	Dextrin
Galactose	Starch
Mannose	Cellulose <i>Mannan</i>
II. Disaccharides, $C_{12}H_{22}O_{11}$	Glycogen
Sucrose	Inulin
Maltose	3. Mixed polysaccharides
Lactose	Gums
Cellobiose	Mucilages

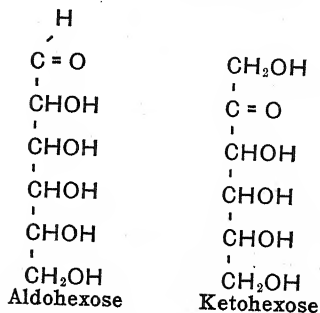
important distinguishing feature of the sugars, many of which are stereoisomers of each other.

#### THE CHEMISTRY OF THE CARBOHYDRATES

Only a few of the *monosaccharides* occur free in nature. Most of them are obtained as hydrolytic or fermentation products of more complex plant constituents. They are often called the simple sugars. All are soluble in water. They are classified into subgroups on the basis of the number of carbon atoms in the chain. There are trioses, tetroses, and others, as well as the pentoses and hexoses given in the table.

**28. Pentoses.**—The pentose sugars have been found as such in small amounts in certain plants, but they occur primarily in a polymerized form in the pentosans. Upon the hydrolysis of hay, oat hulls, corn cobs, and many woods, *xylose* is produced. *Arabinose* is obtained from gum arabic and other gums. *Ribose* is found in some nucleic acids (Sec. 84) and in the vitamin flavin (Sec. 166).

**29. Hexoses.**—The hexoses comprise a large group of sugars, several of which play a significant role in nutrition either as components of foods or as products of metabolism in the body. Glucose and fructose are the only ones which occur free in nature. The hexoses are divided into aldoses and ketoses according to whether they contain aldehyde or ketone groups. Thus glucose is an aldo-sugar, while fructose is a keto-sugar. The general formulas for the two classes of hexoses are given below:



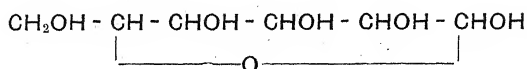
It is noted that each has five hydroxyl groups. The number of stereoisomers which can occur among the sugars is evident from these formulas. The formula for aldohexose has 4 asymmetric carbon atoms and thus 16 stereoisomers are theoretically possible. Of these, 4 optically active forms are known to occur in nature, and the others have been prepared in the laboratory. Eight stereoisomers of ketohexoses are theoretically possible of which two have been found in nature. The physiologically important hexoses are glucose, fructose, galactose, and mannose.

The 16 stereoisomeric aldohexoses consist of 8 pairs of mirror images of each other, *viz.*, *d*-glucose and *l*-glucose, *d*-galactose and *l*-galactose, and 6 other pairs. Similarly, the 8 ketohexoses occur as 4 pairs of mirror images. The letter prefixes denote a conventional basic structural configuration and not direction of rotation. *d*-Glucose is dextrorotatory, but *d*-fructose is levorotatory. This usage of the letters, which seems to be fixed, is unfortunate because it results in confusion. For a more complete discussion of stereoisomerism in the sugars, the student is referred to a textbook of physiological chemistry.

Sugars containing an aldehyde or ketone group have a reducing power which classes them as *reducing sugars*. For example, when they are boiled with an alkaline solution of copper sulfate, such as Fehling solution, the cupric ion is reduced to a cuprous ion present as the oxide, a brick-red precipitate. This is an important reaction which is made use of for both qualitative tests and quantitative determinations. The reaction is not specific, however, because it is given by other reducing substances.

The *glucose* which occurs in nature has the *d* configuration, and it has a specific rotation of  $+52.5^\circ$  which accounts for its other name, *dextrose*. When dissolved *d*-glucose occurs in cyclic

forms of which the amylene-oxide or 1,5-lactone form is written as follows:



There are two stereoisomers represented by this formula which are called  $\alpha$ - and  $\beta$ -*d*-glucose. It is agreed that *d*-glucose can exist also as a 1,4-lactone, and there is evidence that this is one of the forms in which it reacts in its nutritional relationships. The formula shows that additional lactone structures are possible.

Glucose is found widely distributed, though in small amounts, in fruits and plant juices and also in honey. It is obtained commercially by the hydrolysis of cornstarch, which is a product of the same process which yields corn-gluten feed and meal for animal feeding. Glucose is of special interest in nutrition, because it is the principal end product of the digestion of higher carbohydrates, the form in which these nutrients circulate in the blood and the form in which they are utilized to furnish energy. It has a sweet taste but is not so sweet as cane sugar. It is fermented by yeast to form ethyl alcohol and carbon dioxide, and it also undergoes an acid fermentation. Glucose is a reducing sugar. Various products arise from its oxidation, notably glucuronic acid which is a detoxicating agent in the body (Sec. 91).

*Glucosides* are etherlike combinations of acids, alcohols, and aldehydes, frequently of the benzene series. As an example, amygdalin, which occurs in bitter almonds, contains glucose combined with benzaldehyde and hydrocyanic acid.

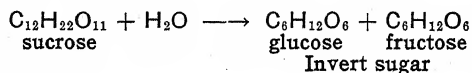
*Galactose* is an aldohexose which occurs in milk sugar in combination with glucose. It is a reducing sugar and has a specific rotation of  $+80^\circ$ . Certain galactosides occur in the brain and nervous tissue.

*Mannose* is an aldohexose occurring in mannans, a group of polysaccharides widely distributed in plants. It has a specific rotation of  $+14.6^\circ$ .

*Fructose* is the only important ketohexose. It occurs free along with glucose in fruits and honey and in combined form in higher carbohydrates. Fructose has a specific rotation of  $-92^\circ$ , which accounts for its other name, *levulose*. It is sweeter than sucrose and readily undergoes fermentation.

**30. Disaccharides.**—The disaccharides derive their name from the fact that they are combinations of two molecules of monosaccharides. Their general formula,  $C_{12}H_{22}O_{11}$ , indicates that one molecule of water is eliminated in the combination. They are soluble in water, though in varying degrees.

*Sucrose* is made up of a combination of one molecule of *d*-glucose and one of *d*-fructose. It occurs in sugar cane and sugar beets and thus is the sugar used on the table and in cooking. It occurs also in ripe fruits, in tree sap (whence maple sugar) and elsewhere in nature. Sucrose has a specific rotation of  $+67^\circ$ , but it is not a reducing sugar. On hydrolysis with the enzyme, *sucrase*, or with dilute acids, sucrose is split into its constituent monosaccharides. The resulting sugar mixture is levorotatory. Since the hydrolysis thus results in a change from a dextro- to a levorotation the process is called *inversion* and the mixture of glucose and fructose is called *invert sugar*.



*Maltose* consists of two molecules of *d*-glucose joined together, as is evident on its hydrolysis by *maltase* or by acids. It derives its name from the fact that it is produced from starch by the action of malt which contains the starch-hydrolyzing enzyme, diastase. Maltose is a reducing sugar and is strongly dextro-rotatory ( $136^\circ$ ).

*Lactose* is the sugar of milk, and it consists of one molecule of glucose and one molecule of galactose, which are produced by hydrolysis with *lactase* or acids. It is a reducing sugar with a specific rotation of  $+55^\circ$ , and it is only one-sixth as sweet as sucrose. Lactose is of special interest in nutrition, because it makes up nearly half of the solids of milk, nature's food for the young, and because it does not occur in nature except as a product of the mammary gland. This sugar has several physiological properties distinguishing it from others. It is less likely than glucose or sucrose to undergo acid fermentation in the stomach, a process which may result in irritation. Lactose promotes acidity in the intestine, favoring the development of desirable types of bacteria, the acidophilic organisms, and opposing the growth of the undesirable putrefactive bacteria. Several studies have shown that this sugar favors calcium and phosphorus

assimilation, probably by increasing their absorption. Lactose is more slowly absorbed than other sugars which results in certain physiological advantages, but it also means that large intakes are likely to cause diarrhea.

*Cellobiose* is a reducing disaccharide which is produced from cellulose by the action of microorganisms, a process which takes place in the digestive tract of herbivora. In the laboratory, cellobiose is obtained from cellulose by acteolysis, *i.e.*, simultaneous acetylation and hydrolysis. It yields glucose on hydrolysis.

**31. Trisaccharides.**—The trisaccharides consist of three monosaccharides, as the name indicates. Raffinose is a trisaccharide which occurs in sugar beets, cottonseed, and elsewhere, and which is composed of glucose, galactose and fructose. On hydrolysis it first yields fructose and the disaccharide, melibiose. The latter, which is isomeric with lactose, is next broken up into glucose and galactose.

**32. Polysaccharides.**—The polysaccharides are complex carbohydrates which are polymerized anhydrides of a large but undetermined number of the simple sugars, as their empirical formulas indicate. The various subgroups are rather ill-defined, and there is a lack of agreement as regards their classification. They are of high molecular weight, are unreactive, and form colloidal solutions for the most part. Upon hydrolysis by acids and enzymes, they are broken down into various intermediate products and finally into their constituent monosaccharides. Quantitatively, they are the most important nutrients in feeds of plant origin.

**33. Starch.**—The reserve material of most plants consists primarily of starch. When this polysaccharide is hydrolyzed with acids or enzymes it is changed into dextrin, maltose, and finally into glucose. In the ripening of fruits, there is a change of starch into sugars. The starches of different plants differ as regards the size and shape of their grains. These properties furnish a means of microscopic identification. Strictly speaking, the various starches are not pure carbohydrates because they contain minute amounts of acid radicals, which are sometimes fatty acids and which sometimes contain phosphorus. Starch gives a characteristic blue color with iodine.

In certain plants, notably the Jerusalem artichoke, *inulin* replaces starch as the reserve material. Inulin is a polysaccharide which yields fructose on hydrolysis.

✓ 34. **Glycogen.**—The small amount of carbohydrate reserve in the animal body exists in the liver and muscles in the form of glycogen, which resembles starch in certain properties as well as in function. It is therefore frequently called "animal starch." Glycogen is present in lower as well as in higher animal life. Toward the end of the larval period, it makes up 33 per cent of the dry weight of bee larvae. It is also present in yeasts and certain other fungi. Differing from starch, glycogen is soluble in water and gives a brown to red color with iodine, but both yield glucose as the sole end product on hydrolysis.

35. **Dextrin.**—This is an ill-defined group of intermediate compounds resulting from the hydrolysis and digestion of starch, and they are also produced from starch by the action of heat. They occur temporarily in both plants and animals as a result of metabolic processes and are particularly abundant in germinating seeds. The dextrins are much more soluble than the starches, and their molecules are certainly much smaller. Like lactose, dextrin furnishes a favorable medium for the development of acidophilic organisms in the digestive tract.

36. **Cellulose.**—The term cellulose is applied to a group of polysaccharides which are more resistant to chemical reagents than is starch. Weak acids and alkalies have little effect on cellulose, but it can be hydrolyzed by strong acids to glucose. It is dissolved by an ammonical copper solution—Schweitzer's reagent. It is not acted upon by any enzyme secreted by mammalian tissues, but bacteria break it down. These are properties which have an important bearing on its usefulness in nutrition. There are many different celluloses in which the number of polymerized glucose molecules has been variously determined to range from 900 to 2000.

Cellulose occurs in a nearly pure form in cotton, a simple cellulose. As the framework of plants and the protective coating of their seeds, it occurs combined with various aromatic derivatives, and as such is called a compound cellulose. Wood is chiefly cellulose combined with lignin, and this combination is called lignocellulose. *Lignin* is a compound of uncertain structure having an aromatic nucleus and three or four free hydroxyl groups and methoxyl groups. Lignocellulose and similar compounds are more resistant to breakdown than is simple cellulose.



**37. Pentosans.**—This group of polysaccharides differs from cellulose in yielding pentose sugars on complete hydrolysis with acids, and most of the group, at least, are much less resistant to acids and alkalis. The pentosans make up about 20 per cent of the complex carbohydrates in hays and occur in lesser proportions in various concentrates, such as the oil-meals. When pentosans are boiled with hydrochloric acid, *furfural*, an aldehyde is produced. This reaction is the basis of the quantitative determination of pentosans, and it is used in the commercial production of furfural from oat hulls and corn cobs.

The pentosans and certain hexosans are commonly classified by the plant chemist as *hemicelluloses*, a term used to designate a group of complex polysaccharides in the cell wall which are much more readily broken down by acid or alkali than is cellulose. Different writers use the term somewhat differently and it cannot be considered to represent a hard and fast distinction.

**38. Mixed Polysaccharides.**—The gums and mucilages represent a heterogeneous group of complex substances which occur in the structures of various plants and which yield various pentose and hexose sugars on hydrolysis. The pectins of fruits may be classified here.

*Chitin* is a carbohydrate derivative occurring in the hard coverings of certain insects. On hydrolysis, it yields glucosamine and acetic acid, and it is considered to be a polymerized mono-acetyl glucosamine.

**39. The Chemical Determination of Carbohydrates.**—In the usual procedure for the analysis of feeds, the carbohydrates are determined as two groups; *crude fiber* and *nitrogen-free extract* (N.F.E.). The separation is obtained by a chemical method devised over seventy-five years ago by Henneberg and Stohmann<sup>1</sup> and known as the Weende method after the name of their experiment station. After the removal of the water and fatty material from a given sample of feed, it is boiled for 30 min. with weak sulfuric acid (1.25 per cent) and then for the same time with

<sup>1</sup> Wilhelm Henneberg (1825–1890) and Friedrich Stohmann (1832–1897) were pioneer German workers in the nutrition of farm animals. Their work included respiration studies, digestion trials, and chemical analyses of feeds and animal tissues. Their early studies, including their work on crude fiber, are reported in their two-volume publication: *Beiträge zur Begründung einer rationellen Fütterung der Wiederkäuer*, vols. I, II, Schwetschke u. Sohn, Brunswick, 1860, 1865.

alkali of the same strength. This procedure removes the proteins, sugars, and starch, leaving as a residue most of the cellulose and other complex polysaccharides along with some mineral material. The loss on ignition of this dried residue is taken as the crude fiber. Since the crude fiber consists primarily of cellulose and other polysaccharides which serve as the structural and protective parts of plants, it is evidently higher in hay and similar roughages than in the grains, and it is higher in those seed by-products which consist largely of the outer coatings, such as wheat bran or oat hulls, than in the seed as a whole.

The nitrogen-free extract, which comprises the sugars, starch, and a large part of the material classed as hemicellulose, is determined by difference. It is represented by the figure obtained when the sum of the water, ash, protein, fat, and crude fiber of a feed is subtracted from 100. Since the figure is determined by difference instead of directly, it includes the cumulative errors of the other determinations and thus is not an exact value. The total error here involved is not a serious one from the standpoint of routine feed analysis and of the use of the value in practice, and thus the indirect procedure is employed in place of the more time-consuming direct determination of the various sugars and starch. Primarily because it contains the starch, nitrogen-free extract makes up the principal constituent of most feeds of plant origin.

Though the Weende method is an empirical one which does not provide any sharp separation into chemical groups, it is useful because it makes a distinction between the more digestible and the less digestible carbohydrates. The distinction is by no means absolute, for crude fiber undergoes a very considerable breakdown in the digestive tract of Herbivora under the action of microorganisms, particularly in ruminants, and it does not remain entirely unattacked in Omnivora (Sec. 41). Further, the nitrogen-free extract includes pentosans and small amounts of other complex polysaccharides which are by no means completely digestible. A recent study by Norman<sup>1</sup> has shown that cellulose is somewhat attacked by the alkali used in the crude-fiber method and that the lignin is partially removed. This finding illustrates the limitations of the method as an indicator of digestibility, since lignified cellulose is known to be especially

<sup>1</sup> NORMAN, A. G., The composition of crude fiber, *J. Agr. Sci.*, **25**, 529-540, 1935.

resistant to breakdown in the digestive tract. Despite these limitations the method continues to be employed, because it is a useful measure of the nutritive value of carbohydrates of plant origin and because of its simplicity. Even in Herbivora, nitrogen-free extract is generally much better digested than is crude fiber.

The chemist has special methods for the determination of the various complex polysaccharides, even as is the case for starch and sugar, but most of them are too time consuming to be applicable to routine feed analysis. Williams and Olmsted<sup>1</sup> have recently published a useful method for determining the lignin, cellulose, and pentosans in feces, and certain suggestions are made for its employment in food analysis. Their method is designed to overcome the limitations of the Henneberg and Stohmann procedure. They refer to several modifications of the latter method which have been used by various investigators.

#### CARBOHYDRATE METABOLISM

From the standpoint of nutrition, two processes are essential for life—the assimilation of food and the removal of waste products. The food consists of complex chemical units, such as proteins and fats; the waste products are simple compounds, such as carbon dioxide and water. The sum of the changes which food undergoes in its conversion to excretory products is called *metabolism*. Some reserve this term for the changes which occur in the absorbed food and which are involved in the breakdown of body tissues, processes which are also referred to as *intermediary metabolism*. These various metabolic changes are governed by physiological processes with which the student is assumed to be familiar in a general way from a previous study of physiology. Thus in the discussions to follow for the carbohydrates and other nutrients these changes as a whole are traced only in outline, as a background for a detailed consideration of certain features which from the standpoint of nutrition require a more extended discussion than they receive in an elementary course in physiology.

**40. The Digestion of Sugars and Starch.**—The previous discussion of the hydrolysis of compound sugars and starch has

<sup>1</sup> WILLIAMS, RAY D., and W. H. OLMSTED, A biochemical method for determining indigestible residue (crude fiber) in feces: lignin, cellulose and non-water-soluble hemicelluloses, *J. Biol. Chem.*, **108**, 653–666, 1935.

indicated the processes by which these carbohydrate groups are broken down to the simple sugars under the action of specific enzymes secreted into the digestive tract. Since starch predominates in most rations, the principal end product is glucose. Where milk is included in the ration, as is always the case for the suckling, galactose is also formed; and fructose is a digestion product of feeds containing sucrose, such as sugar beets. These digestive processes are common to all animal species including man, and their physiology requires no special discussion from the standpoint of nutrition. The breakdown of the higher carbohydrates, however, presents certain features, variable with the species, which have important bearings on the nutritive value of the ration as a whole and which need more extended consideration.

**41. The Digestion of Complex Polysaccharides.**—The breakdown of cellulose and similar compounds is accomplished, not by enzymes secreted into the digestive tract but rather by enzymes of symbiotic microorganisms. Of these organisms, bacteria and flagellate infusoria are the most important. This symbiotic relationship occurs widely in animals which live on plant food, but it is developed to the highest degree in ruminants since the rumen provides both the capacity and other factors which are most favorable to its activity. The symbiosis takes place also in the intestine. In the nonruminant Herbivora the caecum is the principal seat of action, but the colon is also concerned to an important degree. In the Omnivora, symbiosis occurs both in the caecum and colon, but the extent of the action is much less than in Herbivora. Cellulose-splitting microorganisms also play a helpful role in digestion in certain insects.

The evident end products of the microbiotic digestion of cellulose and other complex polysaccharides are gases and acids. The gases are carbon dioxide, methane, and hydrogen, and the acids are acetic, lactic, butyric, isobutyric, propionic, succinic, and others. These same gases and acids, however, can be produced from the fermentation of simple sugars by bacteria, and, thus, the unique role of microorganisms in cellulose digestion is concerned with its initial breakdown. Woodman and Stewart<sup>1</sup>

<sup>1</sup> WOODMAN, H. E., and J. STEWART, The mechanism of cellulose digestion in the ruminant organism. II. The transformation of cellulose into glucose by the agency of cellulose-splitting bacteria, *J. Agr. Sci.*, **18**, 713-723, 1928; III. The action of cellulose-splitting bacteria on the fibre of certain typical feeding stuffs; *ibid.*, **22**, 527-547, 1932.

have produced evidence that this initial breakdown results in the formation of cellobiose which is in turn hydrolyzed into glucose and that the gases and acids result from the fermentation of this sugar. They believe that in the ruminant the major portion of the decomposed cellulose is absorbed as glucose and that the portion which goes to acids and gases is relatively small. According to these findings, decomposition products of the higher polysaccharides in the digestive tract are similar to those from the simpler carbohydrates, though the agencies concerned are different; for it must be considered that the glucose produced from starch is as susceptible to fermentation as that from cellulose.

The gases which are produced from carbohydrate fermentation are a loss to the organism though they are not accounted for as undigested residues, but the acids may be absorbed and serve the body as a source of energy as well as for other purposes. The acids may also be used in the growth of the bacteria themselves. In support of their view that the major portion of the decomposed cellulose is absorbed as sugar, Woodman and Stewart point out that this concept is in keeping with the observation of Kellner (Sec. 199) that digestible fiber has a fattening value similar to starch or sugar, a result which could not occur if the end products of cellulose breakdown were principally gases and acids. In the digestible-nutrient system (Sec. 190) of computing rations for farm animals, digestible fiber is given the same value as other digestible carbohydrates, but this system does not take account of certain other possible differences, notably heat losses (Sec. 204).

**42. Factors Governing the Digestion of Crude Fiber.**—The quantitative relations involved in the microbiotic decomposition of carbohydrates require further study. It is clear that they are variable according to the kind and number of the microorganisms present, which in turn are under the influence of the character of the food. It has been shown, for example, that the addition of easily digestible carbohydrates, such as starch, cane sugar, or molasses to the ration of cattle reduced the digestibility of the fiber, and this observation has been explained on the ground that the bacteria attack the simpler carbohydrates by preference. It is clear that such a shift in substance attacked would lower the nutritive value of the entire carbohydrate portion of the ration, in that less crude fiber would be digested and more of the absorb-

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able sugar would be lost as gases. An illustration is here furnished of one of the factors which may influence carbohydrate digestion, especially in Herbivora, *viz.*, the nature of the microbiotic action.

The differences in the extent of crude-fiber digestion in the various animal species are readily explainable on the basis of the varying opportunities presented for the action of microorganisms. Ruminants are able to digest at least 50 per cent of the crude fiber of most feeds and other Herbivora can do nearly as well, in contrast to the Omnivora which have only a limited ability to digest the complex polysaccharides. Studies by Mitchell and Hamilton<sup>1</sup> have shown that the fiber of oat hulls and alfalfa hay was only 2 per cent digested by swine. Pure cellulose was digested to the extent of 3.5 per cent on the average. Some studies with Carnivora, such as dogs, have given entirely negative results, while other investigations have shown that limited amounts of the higher polysaccharides may be digested by this species. The differences in the results obtained may be due to differences in bacterial flora in the experimental animals. Fiber digestion occurs to a certain extent in the caeca of fowls. These species differences explain why hay and other roughages can form such a large part of the feed of horses and cows and yet be useful in only very limited amounts in the rations of pigs and chickens.

For a given species and animal, there are differences in the degree of the breakdown of crude fiber from different sources which are intimately associated with its chemical and physical nature. The complex polysaccharides of mature plants are less well digested than they are in young, growing plants. The crude fiber of growing pasture grass, fresh or dried, is more digestible than that of hay. Early cut hay is more digestible than hay cut in late bloom or in seed. The difference is due to both chemical and physical structure and particularly to the presence of certain encrusting substances, notably lignin, which are deposited in the cell wall with age. Microorganisms have little or no action on lignin, particularly in mature plants. More important, lignin

<sup>1</sup> MITCHELL, H. H., and T. S. HAMILTON, True and apparent digestibility of oat hulls and alfalfa meal by swine, with special reference to the ability of swine to digest cellulose and crude fiber, *J. Agr. Research*, **47**, 425-435, 1933.

protects the cellulose and other complex carbohydrates against the action of these organisms.

Differences in crude-fiber digestibility have an influence on the digestibility of all nutrients because intact fiber hinders the action of the digesting enzymes on the other nutrients. This is true not only for the various plant parts, such as the stems and seeds with their protective coatings, but it is also true for each cell with its fibrous membrane. Only the tenderest of these membranes are permeable to the enzymes without previous decomposition or rupture. Of course, cellulose-splitting organisms are not the only factors here involved in the destruction of protective coatings, for the cell membrane can be ruptured by the mechanical processes of digestion or softened and disintegrated by chemical action in the digestive tract. But microbiotic action plays a very large role in connection with the fibrous rations of Herbivora, and, thus, the nature and amount of the crude fiber present are important factors governing the extent of the digestibility of the various nutrients in the ration.

While many attempts to isolate from mammalian tissues enzymes which will digest the complex polysaccharides have given only negative results, Iwata<sup>1</sup> in an extensive study has reported the presence of a xylanase in the tissues of the intestine of several Omnivora and Herbivora. He agrees, however, that the digestive action of this enzyme is slight compared to that of microorganisms. Present knowledge suggests that the pentosans and other complex polysaccharides included in the nitrogen-free extract by the Weende method (Sec. 39) behave more like cellulose than starch as regards susceptibility to breakdown in the digestive tract. Further studies of this question for various species are highly desirable.

The literature relative to the digestion of crude fiber in various species has been recently reviewed in a thorough manner by Mangold.<sup>2</sup>

<sup>1</sup> IWATA, HISAIJOSHI, Biochemical studies on xylan, *Imp. Coll. Agr. Forestry (Morioka, Japan) Bull.* 21, 1935.

<sup>2</sup> MANGOLD, ERNEST, The digestion and utilization of crude fiber, *Nutrition Abs. Rev.*, 3, 647-656, 1934. Ernest Mangold is professor of animal physiology and director of the Tierphysiologischen Instituts der Landwirtschaftlichen Hochschule at Berlin. He is the author of a very comprehensive and authoritative work: "Handbuch der Ernährung und des Stoffwechsels der Landwirtschaftlichen Nutztiere, Grundlagen der Fütterungslehre,"

**43. Bulk.**—Crude-fiber content is an important factor governing the bulk of a ration, whatever may be the significance implied in the rather variable usage of this term. As used in connection with a grain mixture the term refers to the weight of a given volume of the feed. For example, oats which weigh approximately 1 lb. to the quart are bulky in contrast to corn meal which weighs  $1\frac{1}{2}$  lb. on the same basis. The bulky concentrates are in general those which are high in crude fiber, although the air spaces between the particles also contribute to bulk. For the ration as a whole, increasing the roughage portion with its high-fiber content increases its bulk. The importance of making up a concentrate mixture so that it will have a certain amount of bulk is stressed by many authorities in order to avoid the formation of a doughlike mass in the stomach which is not readily attacked by the digestive juices. The physiological evidence for this point of view, however, is not entirely conclusive as is apparent from the recent work of Moore, Huffman, and Plum.<sup>1</sup>

Bulk is also considered important from the standpoint that a certain distention of the digestive tract is desirable for the tract's most effective functioning, particularly in the elimination of the feed residues. Of course, this distention can be brought about by a large intake of any kind of food, but it is particularly accomplished for the tract as a whole by indigestible material such as crude fiber. In fact, in human nutrition the term, roughage, is used synonymously with bulk to denote the indigestible portion of the diet. Procter and Wright<sup>2</sup> have advanced the view that water-absorbing capacity is the property of a feed most important in determining its bulk in the digestive tract. Some fibrous materials, such as agar, absorb large quantities of water, while others, such as regenerated cellulose, do not. Linseed-oil meal which is much lower in fiber than wheat bran absorbs three times as much water and, thus, is a more bulky feed in the digestive tract according to Procter and Wright.

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published in four volumes by J. Springer, Berlin, 1929-1932. These volumes constitute an invaluable source of information for students of animal physiology and nutrition.

<sup>1</sup> MOORE, L. A., C. F. HUFFMAN, and M. M. PLUM, Bulk as a factor in formulating grain mixtures for dairy cattle, *J. Agr. Research*, **44**, 789-796, 1932.

<sup>2</sup> PROCTER, FRANK, and N. C. WRIGHT, Bulk in animal feeding, *J. Agr. Sci.*, **17**, 392-406, 1927.



The influence of bulk in promoting the elimination of feed residues is essentially a laxative effect. It is recognized that feeds high in crude fiber tend to be laxative and that a fiber which readily absorbs water and swells is more laxative than one that does not, at least for certain species. A nonfibrous feed which absorbs a large amount of water is less effective, because it is largely digested and thus does not reach the portion of the tract occupied primarily by feed residues. Of course, bulk is not the sole cause of laxative effect, for many feeds are laxative because of specific chemical substances contained in them which promote peristalsis. In certain species including man, large intakes of fiber cause intestinal irritation and other gastrointestinal troubles.

The degree of bulk which is desirable naturally depends upon the species, in view of their variability as regards size and anatomy of their digestive tracts. It is also dependent upon the level of production sought. Too much bulk lessens the consumption of digestible nutrients, and, thus, the intake of bulky material of low digestibility must be limited. Though alfalfa may be ideal as a sole ration from the standpoint of promoting the normal activity of the digestive tract, high producing cows cannot consume enough of it to meet their needs for nutrients. On the other hand, a high intake of a ration too low in bulk may result in indigestion and in the animal going "off feed."

In this connection, it is interesting to note that Mead and Goss<sup>1</sup> have reared heifers on a roughage-free diet from birth to over 18 months of age. The animals were normal in size for the breed and showed no abnormalities other than frequent bloating and lack of regular rumination. With the exception of crude fiber, they digested the nutrients of their ration as well as did animals reared on the same ration plus wood pulp as an artificial roughage. Rumination did not occur any more regularly in the animals receiving the wood pulp than in the other group. The question of the optimum amount of roughage in the ration from the standpoint of its most effective utilization needs further study.

**44. The Glycogenic Function of the Liver.**—The absorbed products of carbohydrate digestion consist of glucose, other

<sup>1</sup> MEAD, S. W., and HAROLD GOSS, Ruminant digestion without roughage, *J. Dairy Sci.*, 18, 163-170, 1935.

monosaccharides such as fructose and galactose depending upon the nature of the diet, and any organic acids which have entered as products of carbohydrate fermentation. These various substances are carried by the portal vein to the liver where they are converted into glycogen, which in turn is gradually reconverted into glucose; the form in which all carbohydrates serve the body. Glycerol and certain amino acids are also converted into glycogen as is discussed later. While the liver is by far the largest single storehouse of glycogen, the latter is found in limited amount in practically all tissues, notably in the muscles. In fact, the total found in all muscles may exceed that present in the liver. The main purpose of this glycogen is to provide an easily available source of energy-producing material for use as needed. Thus each tissue may have a small store, but the liver contains the main supply which, when thrown into the general circulation as sugar, becomes available for use by any tissue in the body.

The glycogenic function of the liver provides a mechanism whereby the blood-sugar level may be held within the comparatively narrow limits compatible with normal metabolism. The blood of cows and sheep contains from 40 to 60 mg. of sugar per 100 cc. In the case of man and the dog, the usual range is 70 to 90 mg. Similar values are obtained for the pig on the average, but much greater variations have been reported. Birds have higher blood-sugar values than do mammals, but cold-blooded animals show very low figures, such as 20 mg. commonly found for the frog.

The temporary storage of glycogen following carbohydrate absorption prevents *hyperglycemia*, i.e., a blood-sugar level above the normal range; and the later release of this glycogen as glucose to balance the withdrawal of sugar from the blood by the tissues prevents the opposite, *hypoglycemia*. This glycogenic function of the liver is under the control of certain hormones, notably epinephrine from the adrenals which accelerates glycogenolysis, and insulin from the pancreas which retards it. If this control fails, either hyperglycemia or hypoglycemia may result.

**45. Transformation of Sugar into Fat.**—The ability of the liver and other tissues to store sugar as glycogen is limited, and, thus, when the carbohydrate intake regularly exceeds the current need of the body for energy purposes, sugar is transformed into

fat. This process takes place on a large scale in the fattening of animals, since their food consists principally of carbohydrates. This formation of body fat from carbohydrate food was first demonstrated by Lawes and Gilbert<sup>1</sup> by means of a slaughter experiment. They chose pigs from the same litter and of the same size. Some of these animals were slaughtered at the start and analyzed as controls, while the others were killed after being fed for an extended period on a low-fat ration of known composition. The data obtained from the analysis of these animals, compared with the data from the controls, showed that the pigs had stored more fat than could have resulted from all of the fat and protein fed and, therefore, that a part of their fat must have been formed from carbohydrates. The formation of milk fat from carbohydrate was demonstrated by Jordan and coworkers<sup>2</sup> in a somewhat similar way by feeding a ration low in fat and showing that the milk fat exceeded that which could have come from the total protein and fat in the food, while the weight and appearance of the animal indicated that the milk fat could not have been made at the expense of body fat.

The formation of fat from carbohydrate is also readily demonstrated by means of the carbon balance (Sec. 198) and by the measurement of gaseous exchange (Sec. 196). The transformation consists of many intermediary steps involving the breakdown of the glucose molecule into simple units and the synthesis of glycerol and fatty acids from these units. The exact mechanism is unknown. While this formation of fat from sugar takes place very readily, it is doubtful whether the reverse process occurs at all. It is recognized that glycerol can be changed into glucose, but the transformation of fatty acids into carbohydrate has not been proved and is denied by many.

<sup>1</sup> See footnote, p. 11.

<sup>2</sup> JORDAN, W. H., and C. G. JENTER, The source of milk fat, *N. Y. Agr. Expt. Sta. Bull.* 132, 1897; JORDAN, W. H., C. G. JENTER and F. D. FULLER, The food source of milk fat; with studies on the nutrition of milch cows, *ibid.*, 197, 1901. Whitman H. Jordan (1851-1931), following service at the Connecticut, Pennsylvania, and Maine Agricultural Experiment Stations, was director of the New York Experiment Station at Geneva for 25 years. As one of the pioneers in the development of Experiment Station work, he championed the view that the maintaining of rigidly scientific investigation was the most useful function of these stations.

**46. The Formation of Tissue Carbohydrates.**—Small amounts of carbohydrates and their derivatives occur as structural elements in certain tissues. Pentoses are constituents of cell nucleic acids, and galactose exists in combination with lipids in nervous tissue. Various carbohydrate groups occur in many conjugated proteins, and cartilage, bones, and tendons contain an amino polysaccharide. The synthesis of these carbohydrate-containing structural elements and their subsequent catabolism represent a phase of carbohydrate metabolism which is little understood.

The formation of lactose from glucose in milk secretion represents a special carbohydrate synthesis which takes place on a large scale (Sec. 305).

**47. The Catabolism of Carbohydrates.**—Glucose is oxidized to furnish energy for body processes, notably muscular activity, and, under certain conditions, it may be oxidized merely to furnish heat. While it is clear that the end products are carbon dioxide and water, there is a chain of intermediate reactions which are not completely understood. The process has been particularly studied in connection with muscle contraction. In the breakdown of muscle glycogen, lactic acid is formed and oxidized to carbon dioxide and water, but the process is much more complicated than is here implied. It involves many intermediary products and side reactions concerning which there is still much debate. Glucose and lactic acid are intermediary products, and phosphoric acid and creatin are intimately associated with certain stages of the process as a whole, and probably the same is true for adenylypyrophosphoric acid. A brief description of the chemical reactions which are considered to take place and of the energy relations involved is given later (Sec. 339).

**48. Diabetes.**—The normal end products of carbohydrate metabolism are carbon dioxide and water which are excreted through the lungs, skin, and urine. In diabetes there is a failure of this metabolism whereby the glucose content of the blood is greatly increased and the sugar is excreted in the urine. Normally the urine contains only traces of sugars, and the occurrence of a measurable amount of glucose in the urine is called *glycosuria*. The accumulation of sugar in the blood in diabetes is due to a lack of insulin, as a result of a failure of its formation in the pancreas. Recent research has shown, however, that diabetes is not purely

of pancreatic origin. Other hormones besides insulin apparently play some role. This failure of carbohydrate metabolism is also accompanied by a failure of the complete oxidation of fats (Sec. 70) resulting in acidosis as another serious feature.

In diabetes the body tissues waste away, the alkali reserve of the blood is decreased by the acidosis, and the body is poisoned by the accumulated, incompletely catabolized products. This disease is very common in man, and it occurs occasionally in dogs, but it is very rare in farm animals. Before the discovery of insulin, diabetes was always fatal, but now the regular injection of insulin into the blood stream provides a way of overcoming its effects, though not of curing it. Overdosage with insulin results in hypoglycemia with consequences equally as serious as those resulting from too high a blood-sugar level. This illustrates the delicacy of the physiological control over carbohydrate metabolism which is essential to health.

The occurrence of glycosuria is not limited to diabetes. Large intakes of sugar may result in such a large and rapid absorption as to exceed temporarily the body mechanism controlling its level in the blood. The resulting hyperglycemia is in turn responsible for sugar excretion in the urine. This is referred to as *alimentary glycosuria*. The kidneys function to remove the excess which would otherwise prove harmful. Thus the kidneys play a role in regulating blood-sugar level. The intake which is necessary to cause this spilling over into the urine varies somewhat with the nature of the sugar, with the rate of absorption, and also with the species of animal. The amount of a given sugar which can be ingested at any one time without causing sugar to appear in the urine is called *sugar tolerance*. Alimentary glycosuria is not a serious matter. In view of the nature of their usual rations, its occurrence in farm animals is not to be expected. Lactosuria, however, sometimes occurs in dairy cows, arising from the reabsorption of lactose in quantity from the mammary gland.

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## CHAPTER V

### THE LIPIDS AND THEIR METABOLISM

Plant and animal materials contain a group of substances, insoluble in water but soluble in ether, chloroform, and benzene, which are most commonly referred to as lipids or lipides. The group includes the fats and several closely related or associated compounds, such as the phosphatides, sterols, and others. From the standpoint of the amounts present in the animal body and its food, the fats are by far the most important members of the group, but several of the other lipids play very significant roles in nutrition and in physiology. As an example, one may cite ergosterol which is a mother substance of vitamin D.

**49. Classification of Lipids.**—In using the term lipids to designate this group of physiologically important compounds, the nomenclature and classification suggested by Bloor<sup>1</sup> are followed (Table V). The term, lipoids, is used instead of lipids by some

TABLE V.—CLASSIFICATION OF THE LIPIDS

Simple Lipids.—Esters of the fatty acids with various alcohols

Fats—esters of the fatty acids with glycerol<sup>1</sup>

Waxes—esters of the fatty acids with alcohols other than glycerol

Compound Lipids.—Esters of the fatty acids containing groups in addition to an alcohol and fatty acid

Phospholipids—substituted fats containing phosphoric acid and nitrogen—*lecithin*, *cephalin*, *sphingomyelin*

Glycolipids—compounds of the fatty acids with a carbohydrate and containing nitrogen but no phosphoric acid—*cerebrosides*

Aminolipids, sulfolipids, etc.—groups which are at present not sufficiently well characterized for classification

Derived Lipids.—Substances derived from the above groups by hydrolysis

Fatty acids of various series

Sterols—mostly large molecular alcohols, found in nature combined with

the fatty acids, and which are soluble in the fat solvents

<sup>1</sup> Commonly called triglycerides or neutral fats.

<sup>1</sup> BLOOR, W. R., Biochemistry of the fats, *Chem. Rev.*, 2, 243-300, 1925-1926.

writers, but this usage is confusing because the same term is also used by others to denote certain members only. Sometimes the term, fat, is employed to include the group as a whole. In addition to their distinctive properties as regards solubility, the lipids are characterized by being esterlike combinations of fatty acids or by being capable of forming such combinations, and they are substances which can perform useful functions in living organisms. These characterizations serve to exclude organic compounds which qualify as regards solubility but which have no biochemical or physiological relationships to the true members of the group.

**50. The Fatty Acids.**—Since the fatty acids are constituents of most of the other lipids, it is helpful to take them up first. A list of those which commonly occur in plant and animal fats is given in Table VI. Melting points are listed for those which are solid above 15°C. All of the acids listed have an even number of carbon atoms since, with a few exceptions, these are the only ones which occur in nature. The saturated acids have the general formula,  $C_nH_{2n}O_2$ , and the melting point rises throughout the series. Butyric acid occurs in milk fat. The acids from caproic to myristic are present in only a few fats, including the fat of milk and the oil of the cocoanut and related plants. Palmitic and stearic acids are widely distributed in plant and animal fats generally. Arachidic and lignoceric acids occur in peanut and certain other vegetable oils. The first four acids listed in the table are classed as volatile since they can be distilled with steam.

The unsaturated acids contain one or more pairs of carbon atoms united by more than one bond. Palmitoleic acid, containing one double bond, occurs in fish oils and also in the body fat of higher animals. Oleic acid, which also has one double bond, is widely distributed in animal and plant fats. Linoleic, also called linolic acid, having two double bonds and linolenic acid with three double bonds are found in the cottonseed, linseed, soybean, and other oil-bearing seeds, as well as in certain animal fats. Arachidonic acid has four double bonds and is found in limited amounts in the fat of various animal tissues. Clupanodonic acid is a highly unsaturated acid occurring in fish oils. These double bonds are reflected in the formulas for the acids, in the smaller number of hydrogen atoms relative to the carbon atoms present. Unsaturated acids have lower melting points and are more reactive than the saturated acids of the same



number of carbon atoms. It is noted, for example, that stearic acid melts at 70°C., whereas oleic, linoleic, and linolenic are liquid at room temperature, although all have 18 carbon atoms. These

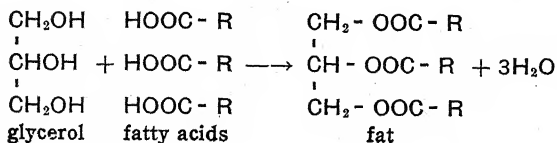
TABLE VI.—FATTY ACIDS COMMONLY FOUND IN LIPIDS

Acids	Formula	Melting point, °C.
Saturated acids:		
Butyric.....	$C_4H_7COOH$	Liquid
Caproic.....	$C_6H_{11}COOH$	Liquid
Caprylic.....	$C_7H_{13}COOH$	16
Capric.....	$C_8H_{15}COOH$	31
Lauric.....	$C_{11}H_{23}COOH$	44
Myristic.....	$C_{13}H_{27}COOH$	54
Palmitic.....	$C_{15}H_{31}COOH$	63
Stearic.....	$C_{17}H_{35}COOH$	70
Arachidic.....	$C_{19}H_{39}COOH$	76
Lignoceric.....	$C_{23}H_{47}COOH$	86
Unsaturated acids:		
Palmitoleic.....	$C_{15}H_{29}COOH$	Liquid
Oleic.....	$C_{17}H_{33}COOH$	
Linoleic.....	$C_{17}H_{31}COOH$	
Linolenic.....	$C_{17}H_{29}COOH$	
Arachidonic.....	$C_{19}H_{31}COOH$	
Clupanodonic.....	$C_{21}H_{33}COOH$	

characteristics are important in physiology because they influence the properties of the fats and other lipids in which the fatty acids are combined.

#### THE FATS AND WAXES

**51. The Structure of Fats.**—The chemical nature of the fats was established in 1814 by the brilliant work of the French chemist, Chevreul. They are esters formed by the union of the trihydroxy alcohol, glycerol, with three molecules of fatty acids. Three molecules of water must be split out, and thus a dehydrating agent is required. Using  $R-COOH$  as the general formula for a fatty acid, the formation of a fat may be illustrated as follows:



The fatty acids may be alike or different. For example, glycerol may combine with three molecules of palmitic acid to form tripalmitin, or it may combine with one molecule each of oleic, palmitic, and stearic acids to form palmito-oleo-stearin. The reaction shown above is a reversible one in that fats may be split into glycerol and fatty acids under the action of dilute mineral acids, enzymes, and steam. The process is essentially a hydrolysis. Enzymatic hydrolysis occurs in digestion. While the term, fat, is employed for all triglycerides, the term, oil, is used in industrial classifications to denote those which are liquid below 20°C. Most vegetable fats fall into the latter class.

The fats which occur in nature consist of triglycerides containing different fatty acids in varying proportions. The principal fatty-acid constituents of most animal fats are palmitic, stearic, and oleic acids, but several others occur in lesser amounts. The fatty-acid distribution in some typical plant and animal fats, as reported by various workers, is given in Table VII. These data

TABLE VII.—PERCENTAGE OF FATTY ACIDS AND PHYSICAL AND CHEMICAL CONSTANTS OF SOME COMMON FATS

	Butter-fat	Lard	Cocoa-nut fat	Soy-bean fat	Corn fat	Cotton-seed fat
<b>I. Saturated acids:</b>						
Butyric.....	3.2					
Caproic.....	1.4					
Caprylic.....	1.6	.....	7.9			
Capric.....	1.8	.....	7.2			
Lauric.....	6.9	.....	48.0			
Myristic.....	22.6	0.7	17.5	.....	.....	0.3
Palmitic.....	19.2	25.2	9.0	6.8	7.3	19.1
Stearic.....	11.4	12.8	2.1	4.4	3.3	1.9
Arachidic.....	.....	.....	.....	0.7	0.4	0.6
<b>II. Unsaturated acids:</b>						
Oleic.....	27.4	54.3	5.7	33.4	43.4	33.1
Linoleic.....	.....	7.1	2.6	51.5	39.1	39.3
Linolenic.....	.....	.....	.....	2.3		
Melting point, °C.....	28-36	35-45	20-25	Liquid at ordinary temp.		
Iodine no.....	26-38	38-44	8-10	130-137	105-125	100-115
Saponification no.....	220-241	193-120	250-260	190-194	87-93	190-200
Reichert-Meissl no.....	23-33	.....	6-8			

must be considered as approximate only, since the amounts of the fatty acids in a given fat are subject to some variation. This is particularly true for the animal fats because their composition is influenced by the diet of the animal. At least some of the fats shown in the table contain small amounts of acids for which no values are given. The fatty-acid make-up of a fat determines its specific chemical and physical properties. Certain constants used for measuring these properties are listed in the last lines of the table. These constants are given as a range since they reflect the variation in fatty-acid distribution. Some samples will fall outside the ranges given.

**52. Melting Point.**—Since a naturally occurring fat consists of a more or less variable mixture of glycerides, its melting point is variable and not sharp. Nevertheless the determination provides a useful measure of hardness. For the fats which are liquid at ordinary temperatures, the *solidifying point* rather than the melting point is generally used as the measure. Although variable according to the make-up of the fat, the temperature of solidification is always lower than that of melting. While the presence of acids of a low molecular weight tends to make for a low melting point, the degree of unsaturation is also an important factor influencing this constant. This is readily evident by comparing the amounts of unsaturated acids in soybean and corn fats, which are liquid at ordinary temperatures and solidify between  $-5$  and  $-10^{\circ}\text{C}.$ , with the amounts in cocoanut fat, which melts at  $20^{\circ}\text{C}.$  or above. Though cocoanut fat has much smaller amounts of unsaturated acids than lard, it melts at a lower temperature because of its high content of acids of low molecular weight.

**53. Iodine Number.**—An unsaturated fat easily unites with iodine, two atoms of this element being added for each double bond. Thus the iodine number is a measure of the degree of unsaturation. It is defined as the number of grams of iodine absorbed by 100 grams of fat, and it is the most useful single measure of the character of fat. The value of 130 for soybean oil, which reflects its high degree of unsaturation, may be contrasted with the figure of 8 for cocoanut oil.

**54. Saponification Number.**—When a fat is boiled with alkali, such as sodium hydroxide, it is split into glycerol and the alkali salt of the fatty acids. These alkali salts are called *soaps* and the

process is called *saponification*. The process occurs in digestion under the action of the sodium salts in the bile. The amount of alkali required to saponify a given amount of fat is a measure of the length of the fatty-acid chains present; for the smaller the fatty-acid molecules the greater is the number of these molecules per gram of fat and, thus, the larger the amount of alkali required for saponification. This measure is called the saponification number. As an illustration, butterfat with its large percentage of the lower fatty acids has a much higher saponification number than corn oil which contains mostly acids with 18 carbon atoms. Saponification splits a mixture of lipids into a *saponifiable fraction* which consists principally of fatty acids, and an *unsaponifiable fraction* or residue which contains the sterols. All naturally occurring fats, such as those listed in Table VII contain some unsaponifiable materials.

**55. Reichert-Meissl Number.**—The determination of the amount of soluble, volatile fatty acids present is a useful measure of the character of butterfat and for detecting adulteration in it, since the large percentages of these acids in butterfat are a distinctive feature. This measure is called the Reichert-Meissl number. The fatty-acid distribution given in Table VII shows why cocoanut fat is the only one, aside from butter, which gives a value of any size. It is also evident that butterfat and cocoanut fat are easily distinguishable by this constant.

Other commonly employed constants are the *refractive index* which measures hardness, and the *acid number* which measures the amount of free fatty acids.

**56. Oxidation of Fats. Rancidity.**—Fats are readily oxidized at the double bond, and, as this occurs, the fat becomes more viscous or harder. This reaction takes place in the drying of paints, involving oxidation of the highly unsaturated acids in the linseed oil which commonly is the carrier of the pigment. Vegetable oils are classified on the basis of their drying properties as nondrying, semidrying, or drying oils. The last include the oils which are highly unsaturated. The rapid oxidation of a highly unsaturated fat generates much heat. Herein lies the cause of the spontaneous combustion of oily rags.

The development of rancidity is the result of hydrolysis and oxidation and perhaps other reactions. Free fatty acids are formed and their oxidation results in the production of aldehydes,

ketones, and acids of lower molecular weight. The changes occur especially in the presence of light, heat, and moisture. Certain substances called *prooxidants*, such as free fatty acids and peroxides, apparently catalyze the process which is thus referred to as *autoxidation*, while certain hydroxy compounds called *anti-oxidants* inhibit it. Rancid fats have disagreeable odors and flavors. In the case of rancid butter, these are due partly to the formation of butyric acid.

**57. Hydrogenation of Fats.**—Double bonds will take on hydrogen as well as oxygen, though less readily. A catalyst is required. This process of hydrogenation produces a saturated and thus a hard fat, out of an unsaturated, soft one. This saturation of the double bonds makes the fat less reactive and thus tends to prevent the oxidative changes of rancidity. Thus hydrogenation is used for improving the keeping qualities of certain fats, especially vegetable oils, used for food, resulting in such products as Crisco. The oils are not completely hydrogenated because if this were done the products would be too hard for convenient use. Completely hydrogenated cottonseed oil melts around 62°C. and gives no iodine number, whereas the partially hydrogenated products used in cooking melt between 35 and 43°C. and have an iodine number of 60 to 75.

**58. Body Fats.**—Terroine<sup>1</sup> classified the body lipids into two groups: the "constant element" and the "variable element." Terroine's distinction was based upon the fact that during periods of inadequate food intake the variable element is drawn upon to furnish energy for body processes, while the constant element remains intact to preserve the essential structures of the body. The constant element represents the part which is essential as a constituent of functioning cells, and consists primarily of phospholipids and sterols. The variable element is the much larger group and represents the fat which has been deposited as an energy reserve. This depot fat consists principally of triglycerides of palmitic, stearic, and oleic acids, with much smaller amounts of various others. The fatty-acid dis-

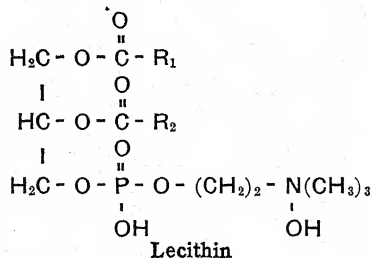
<sup>1</sup> TERROINE, EMILE F., Contribution à la connaissance de la physiologie des substances grasses et lipoidiques, *Ann. sci. nat. Zool.* 4 (10), 5-397, 1920. Terroine is director of the Institut de Physiologie Générale at the University of Strasbourg. He has made many outstanding contributions in the fields of energy and protein metabolism and in comparative physiology.

tribution varies with the species. Cold-blooded animals have softer, and thus more unsaturated, fats than warm-blooded animals. In general, Carnivora have softer fats than Herbivora. Terroine has given a detailed discussion of these species differences. For a given animal, there are also certain differences according to the location of the fat depot. Although a given species tends to deposit a fat of constant composition, the nature of the diet has a marked influence, as is discussed later.

**59. Waxes.**—When a fatty acid combines with one of the higher monohydroxy alcohols instead of trihydroxy glycerol, a wax results. For example, palmitic acid is combined with myricyl alcohol,  $C_{30}H_{61}OH$ , in beeswax and with cetyl alcohol,  $C_{16}H_{33}OH$ , in spermaceti. Waxes have high melting points and are difficult to saponify. They occur as secretions or excretions in many animals, particularly insects, and in many forms of plant life, including bacteria. In plants they form protective coatings to keep out water. They are resistant to the action of digestive juices, particularly because they are not easily saponified.

#### COMPOUND LIPIDS

**60. Phospholipids.**—As their name indicates, the phospholipids, also called phosphatides and phospholipins, are lipids containing phosphorus. They are present in every plant and animal cell and very evidently play a fundamental role in cellular structure and activity. All of them on hydrolysis yield fatty acids and phosphoric acid, and most of them yield glycerol and a nitrogenous base. One group of phospholipids are called *lecithins*, the general formula for which is given below:



$R_1$  and  $R_2$  represent the residues of the molecules of fatty acids. Thus the lecithins may be considered as fats in which one of the fatty acids has been replaced by phosphoric acid and the nitrog-

enous base, choline. The various members of the group contain different combinations of fatty acids. Though lecithins which contain two saturated acids are known, most of them contain at least one unsaturated acid and several contain two. Eggs and liver are particularly rich in lecithins.

The *cephalins* are phospholipids which are similar to the lecithins with the exception that they contain amino-ethyl alcohol (hydroxy-ethyl amine) in place of choline. The *sphingomyelins* yield on hydrolysis, fatty acids, choline, phosphoric acid, and the complex nitrogenous base, sphingosine. They contain no glycerol.

Because of their preponderance of unsaturated fatty acids, the phospholipids of animal tissues are in general much more unsaturated than is the case for the neutral fats. This has naturally led to the view that the functions of the phospholipids are associated in some way with their high degree of unsaturation, a property which would tend to make them more reactive than the triglycerides. It appears significant that fatty acids in active tissues are present principally as phospholipids while the inactive adipose tissues consist primarily of neutral fat. According to the commonly accepted concept of fat metabolism, neutral fats undergo desaturation and are then changed to phospholipids, as preliminary steps in their oxidation. The phospholipids absorb relatively larger amounts of water than do neutral fats and thus they are more readily dispersed in the body fluids. This has suggested that they are the form in which fats are transported in the blood and other fluids.

Phospholipids are found in both the seeds and leaves of plants. In the seeds they probably represent reserve material, since they disappear during germination, but, in the leaves, they are evidently an integral part of the protoplasm.

The *glycolipids* derive their name from the fact that they contain a carbohydrate group, which is galactose. They also contain a fatty acid and sphingosine. They are found particularly in the brain and thus are frequently called *cerebrosides*.

### THE STEROLS

The sterols are alcohols of high molecular weight. They are the unsaponifiable constituents of lipids. In animal and plant tissues, they occur both free and combined with fatty acids as esters.

**61. Cholesterol.**—The most important sterol in animal tissues is cholesterol, and it is doubtless an essential cell constituent. This sterol has the formula,  $C_{27}H_{45}OH$ , and contains one double bond. It occurs in both the free and combined state in the blood. Though this sterol is being constantly formed and destroyed in the body, its functions remain little known, despite much study. It probably originates in the cells in which it is found. In the blood the fatty acids combined with cholesterol are very unsaturated, and thus it is suggested that the acids so combined may have an activity comparable to those present in phospholipids, with the limitation that cholesterol esters are more difficult to saponify. The actual evidence for the transport of fatty acids by cholesterol is as strong as for their transport as phospholipids.

*Coprosterol*,  $C_{27}H_{47}OH$ , is a sterol found in the feces which is a product of the reducing action of bacteria in the lower intestine.

**62. Phytosterols.**—The sterols of plants are called phytosterols, of which there are several; for example, sitosterol, present in corn. They are probably end products of plant metabolism.

*Ergosterol*,  $C_{28}H_{43}OH$ , is the only sterol which occurs in both plants and animals. It is found in plants associated with phytosterols and in animals, with cholesterol. Ergosterol is of outstanding interest because it is a provitamin of vitamin D (Sec. 152).

The sterols belong to the unsaponifiable fraction of lipids. While this fraction is relatively small in most animal and vegetable fats, some fish-liver oils are very rich in it, notably the liver oil of certain sharks which contains 90 per cent of unsaponifiable matter. Aside from ergosterol, no sterols found in plants are appreciably absorbed into the animal body, but cholesterol is readily absorbed from animal products.

**63. The Determination of Lipids.**—In routine feed analysis, the lipids are determined as *ether extract*. The feed is dried to a moisture-free basis and then extracted for 16 hr. with anhydrous ethyl ether. The extract is weighed after the evaporation of the ether. In addition to lipids, ether extracts plant pigments, such as chlorophyll, xanthophyll, and carotene, and traces of various other substances. Ether also removes certain *essential oils* which are nonlipid products consisting primarily of aromatic esters, aldehydes, and ethers. Thus the use of the term, ether



extract, synonymously with fat, in speaking of the nutrient composition of feeds and rations, is not strictly accurate. In certain leafy materials, the amount of ether extract other than esters of fatty acids may represent 25 to 40 per cent of the total. In those foods, however, which we recognize to be the chief sources of dietary fat, namely seeds and animal products, the ether extract consists very largely of triglycerides.

There are special methods for the determination of the various members of the lipids and such determinations are essential for many studies of fat metabolism. The reader is referred to a textbook of physiological chemistry for a discussion of these methods.

### LIPID METABOLISM

Though, following the nursing stage, the lipids make up only a small part of the diet of most animals, the metabolism of fat is of great importance in nutrition, both because of the vital roles played by specific lipids and also because of the extensive fat formation which occurs in the body in fat deposition, in the secretion of milk, and in other functions. Lipids occur as essential constituents in every cell in the body. While the depot fat serves primarily as a source of energy, that deposited under the skin serves also as a nonconducting layer which prevents the too rapid escape of body heat and that around the viscera and certain other organs performs a supporting function.

**64. The Digestion of Lipids.**—Since the lipids of most feeds consist to a very large extent of triglycerides, the products of lipid digestion are essentially the fatty acids and glycerol resulting from the hydrolytic action of the intestinal lipase, which is aided by the saponifying and emulsifying action of the bile. To the extent that compound lipids are present, there arise in addition small amounts of other products such as phosphoric acid and nitrogen bases, but the free sterols are not acted upon in the digestive tract. The sterol esters may be hydrolyzed, setting free their fatty acids. From a quantitative standpoint these free and combined sterols are a negligible part of the ration. Of the plant pigments which make up a sizable fraction of the ether extract of certain feeds, notably leafy material, some, such as chlorophyll, are destroyed in the digestive tract while others are not attacked.

**65. Fecal Lipids.**—The ether extract of the feces consists of digestible fats which have escaped the action of the digestive juices, lipids which are not absorbable such as plant sterols, and nonlipid material of food origin such as pigments. It is recognized that the feces may also contain *metabolic fat*, which is ether-soluble fecal material of body origin such as the residues of digestive juices, as distinguished from undigested or unabsorbed food lipids. The ether extract of the feces of Carnivora and Omnivora shows that the lipids of the rations commonly fed these species are nearly completely digested. It is considered that a sizable portion of this extract represents metabolic fat, though the question has been extensively studied only in the dog. In the case of Herbivora, fat digestion is much less complete, owing primarily to the protective action of undigested cellulose surrounding the fat, which serves as a barrier against digestive action in general. The ether extract of the food of Herbivora also contains relatively more nonabsorbable material such as pigments.

Several recent studies have dealt with the question of the relation of melting point and degree of saturation to the digestibility of fats, but the data as a whole are not sufficiently in agreement to provide a definite answer. Very few experiments have been carried out with farm animals.

**66. The Absorption of Lipids.**—According to the generally accepted theory, the principal products of lipid digestion, namely the fatty acids and glycerol, are absorbed as such and then reformed into fat. At least 60 per cent of this absorbed fat enters the general circulation by way of the thoracic duct, and a part is known to pass through the portal circulation, but there is a balance which remains unaccounted for. The absorption of fat results in a rise in the blood lipids which is particularly marked in Carnivora and Omnivora following a meal rich in fat. In Herbivora on their customary rations, no rise is detectable because of the low fat intake and its gradual digestion and absorption. The alimentary fat which is poured into the blood during absorption gradually disappears into the tissues, whence it is used to build structural tissue, deposited as a reserve of energy or used currently for energy purposes.

**67. Fat Deposition.**—The depot fat is formed not only from ingested fat but also from carbohydrates (Sec. 45) and sometimes from protein. Proof that depot fat can arise from food fat is

obtained by feeding triglycerides of certain fatty acids which do not normally occur in the body and identifying these acids in the adipose tissue. The technique of Gage and Fish<sup>1</sup> involving the feeding of fats stained with Sudan III is particularly useful for following the course of ingested fat. This dye is so firmly attached to the fatty acids that it is not removed by the hydrolysis and saponification which occur in digestion, and thus it serves to identify the fatty acids after absorption until they are

TABLE VIII.—COMPOSITION AND PHYSICAL CONSTANTS OF FAT DEPOSITS IN THE STEER<sup>1</sup>

Samples	Water, per cent	Nitro- gen, per cent	Iodine value	Melting point, °C.	Saponi- fication number
Kidney fat.....	4.5	0.18	40.8	45.0	197
Offal fat.....	9.5	0.27	38.4	45.2	196
Loin fat.....	9.1	0.40	43.6	42.0	193
Inside chuck fat.....	11.2	0.31	46.9	40.5	198
Rib fat.....	10.7	0.45	45.2	40.3	199
Round and rump fat.....	14.4	0.62	46.3	39.6	194
Outside rump fat.....	10.9	0.40	49.3	35.2	196

<sup>1</sup> These data are taken in a condensed form from Table 57 in: TROWBRIDGE, P. F., C. R. MOULTON, and L. D. HAIGH, Composition of the beef animal and energy cost of fattening, *Mo. Agr. Expt. Sta. Res. Bull.* 30, 1919.

catabolized. Fat so stained is found in the depots, in the milk secreted by certain species, and in the eggs of the laying hen. For some unknown reason, fats stained with Sudan III do not appear in the milk of the cow.

Approximately 50 per cent of the adipose tissue is found under the skin, *i.e.*, subcutaneous fat. The balance is located around certain organs, notably the kidneys, in the membranes surrounding the intestines, in the muscles, and elsewhere. The Danish scientists, Henriques and Hansen,<sup>2</sup> were pioneers in the study of fat deposition. One of their articles published in 1901, reports a study of the kind of fat deposited in various portions of the body

<sup>1</sup> GAGE, SIMON HENRY, and PIERRE AUGUSTINE FISH, Fat digestion, absorption, and assimilation in men and animals as determined by the dark-field microscope, and a fat-soluble dye, *Am. J. Anat.*, **34**, 1-85, 1924.

<sup>2</sup> HENRIQUES, V., and C. HANSEN, Vergleichende Untersuchungen über die chemische Zusammensetzung des thierischen Fettes, *Skand. Arch. Physiol.*, **11**, 151-165, 1901.

of the dog. The data in Table VIII give the composition and physical constants of the fat deposited in various parts of the steer. The different deposits contain a variable amount of water which usually does not exceed 20 per cent. The nitrogen is present as protein in connective tissue. Deposited fat may contain from 1 to 4 per cent of protein. It also contains 0.1 to 0.2 per cent of mineral matter. The physical constants show that the character of the fat differs somewhat in different locations.

Since adipose tissue always contains some water, it is evident that fat deposition involves a deposition of water also. With a ration rich in fat, there is some retention of water in all tissues including the blood. Fat deposits are considered to be water-in-oil emulsions, in which albumin, lecithin, or soaps act as the emulsifying agent. When the depots are called upon to furnish energy, there may be a retention of water in place of the fat. This has been clearly shown for the human subject by Newburgh and Johnston.<sup>1</sup> By taking account of the water intake and outgo, as well as the energy metabolism, they found that obese individuals frequently maintained or even increased their weight temporarily on a reducing diet, because water was being stored despite the fact that depot fat was being used up. Particularly striking is the observation of Trowbridge<sup>2</sup> that the kidney fat of a steer on a submaintenance ration for 11 months contained 81.4 per cent of water, 9.6 per cent of protein, and only 4.6 per cent of fat, whereas for a check animal the figures were 5.5, 1.7, and 93.1 per cent, respectively. These observations illustrate the limitations of the weight measure as the sole criterion of nutritive state in maintenance or in fattening. The fat-water relationships in adipose tissue may have a bearing on the amount of "shrink" in animals rapidly fattened for market.

**68. Relation of Food Fat to Depot Fat.**—The fact that the nature of the depot fat is markedly influenced by the character of its food source is of large practical importance, since the degree of hardness of this fat is a considerable factor in the market value of the carcasses of meat animals. This is particularly true for hogs as indicated by the "soft pork" problem. The influence of

<sup>1</sup> NEWBURGH, L. H., and M. W. JOHNSTON, Endogenous obesity—a misconception, *J. Am. Dietetic Assoc.*, 5, 275-285, 1930.

<sup>2</sup> TROWBRIDGE, P. F., The resorption of fat, *Am. Soc. Ann. Nutrition Proc.*, 1910, pp. 13-20.

the kind of fat fed upon the character of the body fat is strikingly shown by the following data obtained by Anderson and Mendel<sup>1</sup> with rats in which the oils listed furnished 60 per cent of the energy intake.

Food fat	Iodine number of food fat	Iodine number of body fat
Soybean oil.....	132	123
Corn oil.....	124	114
Cottonseed oil.....	108	107
Peanut oil.....	102	98
Lard.....	63	72
Butterfat.....	36	56
Cocoonut oil.....	8	35

Anderson and Mendel found that the iodine numbers of body fat deposited from various carbohydrates and proteins fell approximately within the range 55 to 70. This range, representing fat synthesized within the body, was thus considered to typify the normal depot fat of the rat. Taking this range as a base line, the data for the various oils given above show the striking influence of large intakes of fats differing widely as regards degree of saturation from that normally deposited. It is noted, however, that the extremes exhibited by the food fats are never reached by the body fats, reflecting the capacity of the organism, in depositing ingested fat, to modify the latter where it is widely different from the normal deposit. Whether this is accomplished by preferential catabolism or by saturation or by desaturation prior to deposition cannot be stated. The data given illustrate the fact that carbohydrates produce a less unsaturated and thus a harder body fat than do most fats found in feeds of vegetable origin.

Many experiments have shown that fat deposition in the hog follows the same principles illustrated above for the rat. The data in Table IX from the work of Ellis and Isbell<sup>2</sup> demonstrate

<sup>1</sup> ANDERSON, WILLIAM E., and LAFAYETTE B. MENDEL, The relation of diet to the quality of the fat produced in the animal body, *J. Biol. Chem.*, **76**, 729-747, 1928.

<sup>2</sup> ELLIS, N. R., and H. S. ISBELL, Soft pork studies. II. The influence of the character of the ration upon the composition of the body fat of hogs; III. The effect of food fat upon body fat, as shown by the separation of the individual fatty acids of the body fat, *J. Biol. Chem.*, **69**, 219-248, 1926.

TABLE IX.—INFLUENCE ON HOG CARCASS OF ADDING VARIOUS OILS TO A BASAL RATION OF CORN AND TANKAGE

Oil supplement	Firmness grade <sup>1</sup>	Melting point, °C.	Iodine number	Fatty Acids		
				Oleic, %	Linoleic, %	Total saturated, %
Peanut oil, 4.1 %.....	MS	34.3	72.4	47.9	13.8	32.5
Cottonseed oil, 4.1 %...	H	45.3	64.4	35.9	15.7	43.0
Soybean oil, 4.1 %.....	MS	31.2	75.7	43.3	18.6	33.8
Corn oil, 4.1 %.....	MS	36.3	76.3	45.0	16.8	33.0
Corn oil, 11.5 %.....	O	24.5	97.2	41.4	31.4	23.1

<sup>1</sup> H = hard, MS = medium soft, O = oily.

the effect of adding various oils to a ration of corn and tankage, which by itself produces a firm fat in hogs. The percentage of fat in these rations is much smaller than that used in obtaining the data previously cited for the rat, and thus the effects are less marked. It is noted, however, that the ration containing cotton-

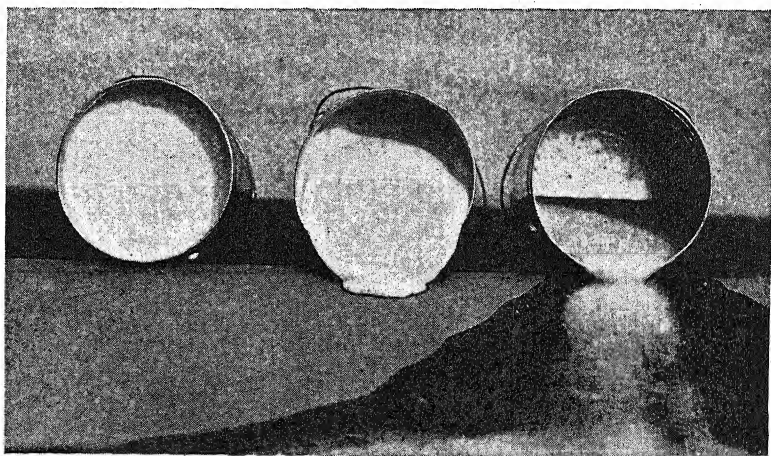


FIG. 5.—Lard from hard, soft, and oily carcasses. (Taken from O. G. Hankins, N. R. Ellis, and J. H. Zeller, *Some results of soft pork investigations*. II, U. S. Dept. Agr. Bull. 1492, 1928.)

seed oil, having the lowest iodine number of the oils used, was the only one which produced a carcass graded as hard. This grade

is reflected in a high melting point, a relatively low iodine number, and the highest percentage of saturated fatty acids. All of the other oils, when added at the same level as the cottonseed, produced medium-soft carcasses, readily explainable by the constants and fatty-acid distribution of their fats. Increasing the level of corn oil produced an oily carcass with fat which melted at room temperature and which consisted of unsaturated acids to the extent of 73 per cent.

The data in Table IX explain why soybeans and peanuts, feeds rich in highly unsaturated fats, can be used in only a limited way for fattening hogs without producing soft pork (Fig. 5). With both hogs and cattle fed the usual fattening rations, the deposited fat becomes harder with age, owing to an increased rate of deposition which results in relatively more of this fat being formed from carbohydrate.

Deposits of soft fat can be modified by a change in diet. When, after a period on feeds rich in unsaturated fat, a ration which will produce a hard fat is given, the deposited fat gradually becomes harder. Ellis<sup>1</sup> has described such a change in hogs, which results when a ration containing peanuts is followed by corn and non-softening supplements. The process is called "hardening off" and is taken advantage of in feeding practice in finishing hogs for market. Anderson and Mendel<sup>2</sup> have shown that the process takes place more rapidly where the animal is fasted for a period before the hardening ration is given.

**69. Food Fat and Milk Fat.**—The character of the food fat has the same influence on the nature of the milk fat as it does on the depot fat. This is shown in Fig. 6, which presents data taken from the work of Maynard, McCay, and Madsen.<sup>3</sup> In this experiment, a ration containing approximately 3.5 per cent of fat on a dry-matter basis was used. In the first and third periods the grain mixture was selected to have a high iodine number, primarily by the inclusion of ground flaxseed, while in the middle period the iodine number was reduced to a low value by the

<sup>1</sup> ELLIS, N. R., Changes in quantity and composition of fat in hogs fed a peanut ration followed by a corn ration, *U. S. Dept. Agr. Tech. Bull.* 368, 1933. (See also earlier papers cited.)

<sup>2</sup> ANDERSON and MENDEL, *loc. cit.*

<sup>3</sup> MAYNARD, L. A., C. M. MCCAY, and L. L. MADSEN, The influence of food fat of varying degrees of unsaturation upon blood lipids and milk fat, *J. Dairy Sci.*, 19, 49-53, 1936.



omission of the flaxseed and the inclusion of cocoanut-oil meal. The curve shows that a change in the iodine number of the food fat from 107 to 43 resulted, in the first 24 hours, in a drop in the value for milk fat from 38 to 32, with a later drop to approximately 26 as the minimum. The restoration of the food fat of high iodine number resulted in a quick rise in the milk-fat value to its level during the first period. These rapid and large changes with a ration which contained only about 3.5 per cent of fat are striking indeed.

In contrast to the marked influence on depot fat and milk, changes in the character of the food fat of the laying hen are not reflected to any extent in the fat of the eggs produced.

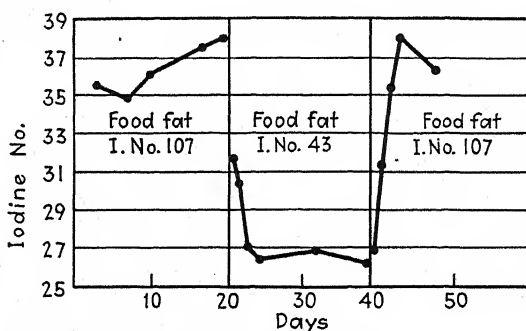
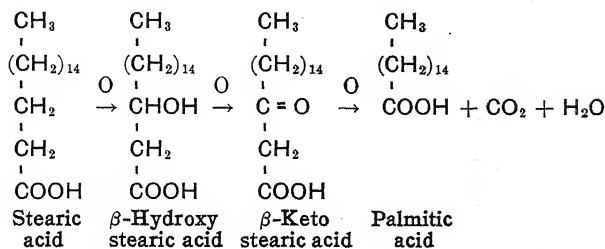


FIG. 6.—The iodine number of milk fat as influenced by food fats of high and low degrees of unsaturation.

**70. The Oxidation of Fats.**—The ultimate catabolic products of fat are normally carbon dioxide and water. Our knowledge of the various stages of the catabolic process is very incomplete. According to the widely accepted theory of Leathes and Raper, the triglycerides, prior to their oxidation, are desaturated and then changed to the more reactive phospholipids, these processes taking place in the liver. Presumably, hydrolysis precedes oxidation with the formation of fatty acids and glycerol, whence the metabolism of the glycerol can follow the same course as the carbohydrates (Sec. 44). It is generally accepted that the essential feature of at least one method of fatty-acid catabolism is  *$\beta$  oxidation*. The fatty acid is oxidized on its beta carbon atom, resulting in the formation of an alcohol, then a ketone, and finally a fatty acid with two less carbon atoms, as follows:





By repetition of this process, the carbon chain is successively broken down by knocking off two carbon atoms at a time until complete oxidation has resulted.

**71. Ketosis.**—Beyond the butyric-acid stage this catabolic process is dependent upon the simultaneous oxidation of glucose, which shows that a certain minimum carbohydrate intake is essential for normal fat catabolism. Thus, under conditions of carbohydrate deprivation or where there is a failure of carbohydrate metabolism as in diabetes (Sec. 48), fat oxidation stops with the formation of the intermediate products of butyric-acid oxidation, *viz.*, hydroxybutyric acid and ketobutyric acid (acetoacetic acid). The latter acid is then converted in part into acetone, with the elimination of carbon dioxide. These three products are called the *acetone bodies*. They accumulate in the tissues and blood, inducing a condition known as ketosis. The increase in these bodies in the blood is referred to as *acetonemia* and their presence in the urine is called *acetonuria*. In Herbivora, however, small amounts of ketone bodies appear to be normal urinary constituents.

Since two of the acetone bodies are rather strong acids, their excessive production uses up the alkali reserve of the blood, producing *acidosis*. The carbon dioxide transporting power of the blood is lessened and cellular oxidation is decreased. This is a serious condition which in extreme cases results in coma and death. This acidosis caused by a failure of fat metabolism is a chronic condition in diabetes. It may occur temporarily in other disturbances of carbohydrate metabolism, or on diets high in fat, either absolutely or relative to their carbohydrate content. It is met sometimes in certain pathological conditions in Herbivora, notably in milk fever and in pregnancy disease in sheep. An increase in ketone bodies also occurs in these diseases and the possibility that they are due to a disturbance of fat metabolism

has been suggested. Carnivora are little susceptible to the disturbance of fat metabolism which results in acidosis, and the same is apparently true for the hog.

Nutrients which favor the development of ketosis, such as fats and certain amino acids which are catabolized similarly to the fatty acids, are referred to as *ketogenic foods*, while carbohydrates and certain other amino acids which promote normal fat metabolism are called *antiketogenic foods*.

The end products of fat catabolism, normally carbon dioxide and water, are excreted through the lungs, kidneys, and skin, even as is the case for the carbohydrates.

**72. Formation of Fat from Protein.**—The previous discussion of fat deposition has indicated that ingested protein can be transformed into body fat. It is known that the nonnitrogenous residue of certain amino acids can be changed into glucose. Since glucose is readily changed into fat, the possibility of the formation of fat from protein is generally accepted. Clearly it is a process which occurs much less commonly, as well as less efficiently, than the change of carbohydrate into fat.

**73. Is Fat an Essential Nutrient?**—Despite the fact that certain lipids are essential constituents of animal tissues, the knowledge that carbohydrates are readily changed into fat and that such essential lipid constituents as phospholipids and cholesterol can be made in the body naturally led to the view that lipids as such are not required in the diet. The recent studies of Burr and Burr<sup>1</sup> suggest that this viewpoint is not absolutely true, at least in the case of the rat. Using diets almost entirely devoid of fats, they found that rats developed a scaly condition of the skin, followed by a necrosis of the tail, a degeneration of the kidneys, a failure of growth, and death. The addition of a small amount of an unsaturated fat or of pure linoleic acid was strikingly effective in preventing or curing these conditions, but saturated acids were ineffective. Certain reproductive failures and an abnormal gaseous exchange (Sec. 196) were also noted on the fat-free diet. On the basis of their results, Burr and Burr postulate that warm-blooded animals in general cannot synthesize

<sup>1</sup> BURR, GEORGE O., and MILDRED M. BURR, A new deficiency disease produced by the rigid exclusion of fat from the diet, *J. Biol. Chem.*, **82**, 345-367, 1929; On the nature and role of the fatty acids essential in nutrition, *ibid.*, **86**, 587-621, 1930.

appreciable quantities of linoleic acid and that the synthesis of other unsaturated acids may be limited also.

Though all of the findings of these investigators are not universally accepted, they serve at least to place fat nutrition in a new light. They also remind us of several findings indicating that fats may have special advantages in the ration beyond that measured by their energy value, even though they may not be absolutely essential. There is evidence that on a low-fat diet the requirement for vitamin B is increased and that the carbohydrate metabolism is unfavorably affected. Mendel and Anderson,<sup>1</sup> in reviewing their various studies with rats, state that an abundant intake of fat was much more effective for storing fat than was an equicaloric intake of carbohydrates.

Since the results reported by Burr and Burr were obtained only by a rigid exclusion of fat from the diet, it seems improbable that a similar injurious effect is to be feared in farm animals in view of the fact that their rations are never devoid of fat or of the specific acids which appear essential. But this must not be interpreted to mean that fat is of no importance in the diet of farm animals. On the contrary, in so far as milk production is concerned, there is clear evidence that a certain amount of fat in the diet is essential for maximum yield, as is discussed later (Sec. 316). As regards fattening in hogs, however, the studies of Ellis and Zeller<sup>2</sup> show no apparent disadvantage from reducing the ether extract of the ration to 0.5 per cent. Despite this very low intake, 124 lb. of fat were stored by a hog in 257 days.

The question of the significance of fat in the rations of farm animals is a subject which deserves more attention from investigators, particularly in view of the recent developments in solvent extraction whereby the oil meals produced contain only a fraction of a per cent of fat, in contrast to the by-products of the old process of pressing out the oil which contain 6 to 8 per cent. Certainly the solvent-extracted products have a lower energy value, and they may be less valuable in other respects, as is indicated by the milk production studies referred to previously.

<sup>1</sup> MENDEL, LAFAYETTE B., and W. E. ANDERSON, Some relations of diet to fat deposition in the body, *Yale J. Biol. Med.*, **3**, 107-137, 1930.

<sup>2</sup> ELLIS, N. R., and J. H. ZELLER, with the technical assistance of S. J. DAHL, Soft pork studies. IV. The influence of a ration low in fat upon the composition of the body fat of hogs, *J. Biol. Chem.*, **89**, 185-197, 1930.

**74. Fat Tolerance in Farm Animals.**—It is a common belief that the Herbivora have a low tolerance for fat, but the experimental evidence on this question is scanty. Certainly the suckling is able to handle a relatively large amount of fat, since milk contains from 25 to 40 per cent of this nutrient on a dry-matter basis. Here we are reminded, however, that Holstein milk is more suitable for raising dairy calves than milk higher in fat. Beyond the milk-feeding stage, Herbivora receive in their normal rations only a small fraction of the fat which they consume as sucklings. While it is probable that certain levels may be expected to cause digestive disturbances and perhaps metabolic disturbances also, it seems unlikely that there need be any concern regarding such effects from any fat level occurring in the commonly fed rations.

Certainly the pig can tolerate rather high intakes of fat, and here no difficulty will arise with the usual feeds, except in respect to soft pork. Hens readily digest rations containing a percentage of fat representing a higher level than found in the commonly fed rations.

It has been frequently reported that meat scraps high in free fatty acids decrease the rate of growth and feed consumption in chicks. According to Schroeder<sup>1</sup> and associates these effects are not due directly to the fatty acids themselves but to the inactivation of vitamin A and, to a lesser degree, of vitamin D in the ration. Apparently the free fatty acids act as prooxidants (Sec. 56) whereby oxidative destruction of the vitamins is rapidly brought about.

**75. Cod-liver-oil Injury in Herbivora.**—In attempting to formulate a satisfactory synthetic diet (Sec. 179) for Herbivora, the Cornell workers<sup>2</sup> noted the production of muscle degeneration

<sup>1</sup> SCHROEDER, CARL H., G. K. REDDING, and L. J. HUBER, Some causes and effects of a high free fatty acid content of meat scraps in poultry rations, *Poultry Sci.*, **15**, 104-114, 1936.

<sup>2</sup> MADSEN, LOUIS L., C. M. MCCAY, and L. A. MAYNARD, Synthetic diets for Herbivora, with special reference to the toxicity of cod-liver oil, *Cornell Agr. Expt. Sta. Mem.* 178, 1935; MADSEN, LOUIS L., The comparative effects of cod-liver oil, cod-liver oil concentrate, lard and cottonseed oil in a synthetic diet on the development of nutritional muscular dystrophy, *J. Nutrition*, **11**, 471-493, 1936; DAVIS, GEORGE KELSO, Further studies in the development of synthetic diets for Herbivora with special reference to cod-liver oil and cod-liver oil concentrates. A thesis presented to the Faculty of the Graduate School of Cornell University, 1937.

in guinea pigs, rabbits, sheep, and goats. The same lesions, though generally milder and less frequent, occurred when the oil was fed with rations of natural feeds, demonstrating that the oil itself was in part responsible for the injury. The harmful agent was found to reside primarily in the saponifiable fraction. While the specific causative factor has not yet been identified, the results are of practical interest because of the use of cod-liver oil as a source of vitamins A and D, though the injury apparently is not due to the vitamins themselves. The levels of intake which were found to cause lesions in sheep lay within the range sometimes recommended to supply the vitamins. In similar experiments with dairy calves, however, these animals were found much more resistant to injury, indicating that the oil could be fed in amounts needed to furnish the vitamins without significant damage. The Cornell workers have not noted this injury in species other than Herbivora. That the rat can tolerate large intakes of cod-liver oil over long periods is well established. The studies have not been extended to other oils except to show that cottonseed oil certainly does not have any such effect. As is discussed later (Sec. 307), cod-liver oil has a specific effect in lowering the fat percentage in the milk of the dairy cow.

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## CHAPTER VI

### THE PROTEINS AND THEIR METABOLISM

Since protein is the principal constituent of the organs and soft structures of the animal body, a liberal and continuous supply is needed in the food throughout life for growth and repair, and thus the transformation of food protein into body protein is a very important part of the nutrition process. The term protein is a collective one which embraces an enormous group of closely related but physiologically distinct members. Plant proteins differ from each other and from animal proteins, each animal species has its own specific proteins and a given animal contains many different ones in its organs, fluids, and other tissues. In fact no two proteins seem to be exactly alike in their physiological behavior. This physiological specificity makes protein nutrition a rather complicated but nevertheless an extremely interesting subject of study.

**76. Elementary Composition of Proteins.**—In common with the fats and carbohydrates, the proteins contain carbon, hydrogen, and oxygen. In addition they contain a large and fairly constant percentage of nitrogen. Most of them also contain sulfur and a few contain phosphorus and iron. The range of *elementary composition* of the more typical proteins is as follows:

	Per Cent
Carbon.....	51.0 to 55.0
Hydrogen.....	6.5 to 7.3
Nitrogen.....	15.5 to 18.0
Oxygen.....	21.5 to 23.5
Sulfur.....	0.3 to 2.2
Phosphorus.....	0.0 to 1.5

They are complex substances, colloidal in nature and of high molecular weight.

**77. Classification of Proteins.**—The various proteins cannot be identified or distinguished from each other by any simple chemical method, and thus their classification is based primarily on physi-

cal properties. The following classification is, in an abbreviated form, the one recommended jointly by the American Physiological Society and the American Society of Biological Chemists:

1. *Simple Proteins*.—This group includes those yielding only amino acids or their derivatives on hydrolysis. It comprises the albumins, globulins, glutelins, alcohol-soluble proteins, albuminoids, histones and protamins. These various subgroups, each of which contains many individual members, are differentiated by solubility in various solvents, such as water, salt solutions and alcohol, and by other characteristics. For example, the albumins, which include lactalbumin, egg albumin, and two albumins of blood serum, are soluble in water and coagulate on heating; while the globulins, such as muscle globulin and legumin of beans and peas, are insoluble in water but coagulated by heat.

2. *Conjugated Proteins*.—Here are included those in which simple proteins are combined with a nonprotein radical. Six subgroups are differentiated.

a. *Nucleoproteins*.—Compounds of one or more protein molecules with nucleic acid. (Present in germs of seed and in glandular tissue.)

b. *Glycoproteins*.—Compounds of the protein molecule and a substance or substances containing a carbohydrate group other than a nucleic acid. (Example, mucin.)

c. *Phosphoproteins*.—Compounds of the protein molecule with a phosphorus-containing substance other than a nucleic acid or lecithin. (Example, casein.)

d. *Hemoglobins*.—Compounds of the protein molecule with hematin or a similar substance. (Example, hemoglobin.)

e. *Lecithoproteins*.—Compounds of the protein molecule with lecithin. (Example, tissue fibrinogen.)

3. *Derived Proteins*.—This group consists of compounds representing altered and degraded products of naturally occurring proteins, produced by the action of heat, enzymes, or chemical agents. This is a large group which is divided into subgroups representing different degrees of degradation as follows: primary protein derivatives, proteans, metaproteins, coagulated proteins, secondary protein derivatives, proteoses, peptones and peptides. The first two members are only slightly altered proteins, while the peptides are comparatively very simple compounds consisting of two or more amino acids which are the ultimate hydrolytic products of all proteins.

78. *Amino Acids*.—At least 23 different amino acids have been identified as constituents of the various plant and animal

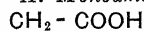


proteins, and an essential distinguishing feature of all of these proteins is recognized to be the kind and amounts of the amino acids present. These amino acids are obtained as the hydrolytic end products when proteins are boiled for many hours with strong acids or when they are acted upon by certain enzymes. They are also the end products of protein digestion and the building stones from which body protein is made, as well as intermediary products in protein catabolism. Thus our study of protein nutrition deals primarily with amino acids.

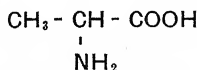
**79. Classification of Amino Acids.**—The amino acids which have been identified in plant and animal proteins are classified according to the series of organic compounds in which they belong, and those in the aliphatic series are further classified according to the number of amino groups and carboxyl groups present. The classification is as follows:

**I. Aliphatic Amino Acids**

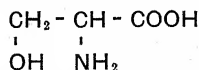
*A. Monoamino-monocarboxylic acids*



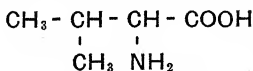
\* Glycine,  $\text{C}_2\text{H}_5\text{NO}_2$   
Amino-acetic acid



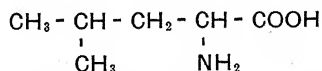
\* Alanine,  $\text{C}_3\text{H}_7\text{NO}_2$   
 $\alpha$ -Amino-propionic acid



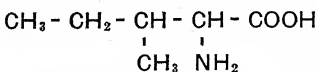
\* Serine,  $\text{C}_3\text{H}_7\text{NO}_3$   
 $\alpha$ -Amino- $\beta$ -hydroxy propionic acid



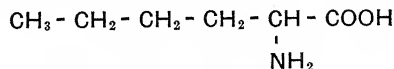
Valine,  $\text{C}_5\text{H}_{11}\text{NO}_2$   
 $\alpha$ -Amino- $\beta$ -methyl butyric acid



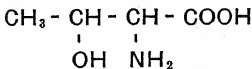
Leucine,  $\text{C}_6\text{H}_{13}\text{NO}_2$   
 $\alpha$ -Amino- $\gamma$ -methyl valeric acid



Isoleucine,  $\text{C}_6\text{H}_{13}\text{NO}_2$   
 $\alpha$ -Amino- $\beta$ -methyl-valeric acid

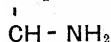
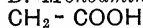


Norleucine,  $\text{C}_6\text{H}_{13}\text{NO}_2$   
 $\alpha$ -Amino-caproic acid

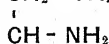
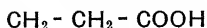


Threonine,  $\text{C}_4\text{H}_9\text{NO}_3$   
 $\alpha$ -Amino- $\beta$ -hydroxy butyric acid

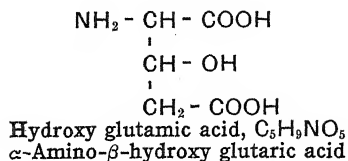
*B. Monoamino-dicarboxylic acids*



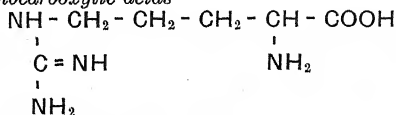
\* Aspartic acid,  $\text{C}_4\text{H}_7\text{NO}_4$   
Amino-succinic acid



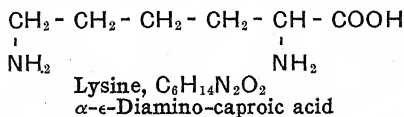
\* Glutamic acid,  $\text{C}_5\text{H}_9\text{NO}_4$   
 $\alpha$ -Amino-glutaric acid



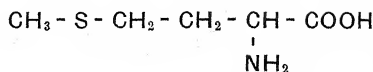
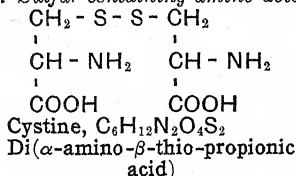
## C. Diamino-monocarboxylic acids



Arginine,  $\text{C}_6\text{H}_{14}\text{N}_4\text{O}_2$   
 $\alpha$ -Amino- $\delta$ -guanidine-valeric acid

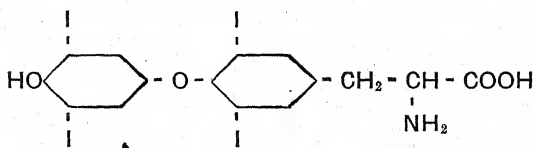
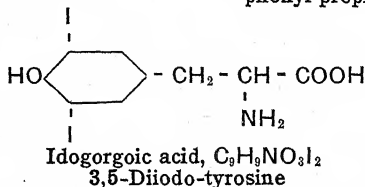
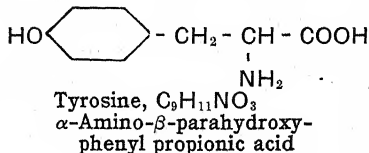
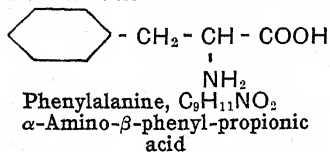


## D. Sulfur-containing amino acids



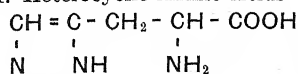
Methionine,  $\text{C}_5\text{H}_{11}\text{NO}_2\text{S}$   
 $\alpha$ -Amino- $\gamma$ -methylthiol-  
 butyric acid

## II. Aromatic Amino Acids

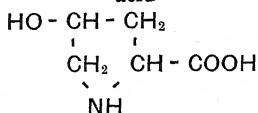


Thyroxine,  $\text{C}_{15}\text{H}_{11}\text{NO}_4\text{I}_4$   
 (3,5, 3',5'-Tetraiodo-4'-hydroxy-diphenyl-ether) alanine

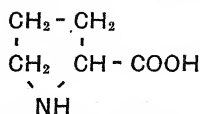
## III. Heterocyclic Amino Acids



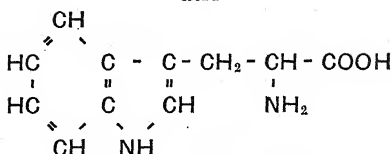
Histidine,  $\text{C}_6\text{H}_9\text{N}_3\text{O}_2$   
 $\alpha$ -Amino- $\beta$ -imidazole-propionic  
 acid



Hydroxy proline;  $\text{C}_5\text{H}_9\text{NO}_3$   
 4-Hydroxy pyrrolidine-2-carboxylic  
 acid



Proline,  $\text{C}_5\text{H}_9\text{NO}_2$   
 Pyrrolidine-2-carboxylic  
 acid



Tryptophane,  $\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}_2$   
 $\alpha$ -Amino- $\beta$ -indole  
 propionic acid

**80. Amino-acid Distribution in Proteins.**—The amino acids are present in widely varying amounts in different proteins, and one or more of them may be entirely absent from many of the proteins. In the quantitative analysis for the amino acids, the chemist hydrolyzes the protein, separates the resulting amino acids into various groups, and then determines the individual members. This is a very difficult procedure and a 100 per cent recovery of all of the acids present is not now obtainable. Thus, though our knowledge of at least the quantitative amino-acid make-up of proteins remains incomplete, approximately accurate data are available for many of the common proteins, as is indicated by the figures in Table X. The incompleteness of the data is indicated by the fact that, if full recovery had been obtained, the figures should add up to considerably more than 100 per cent in all cases, owing to the water added in hydrolysis. Mitchell and Hamilton<sup>1</sup> have tabulated data on the content of several of the amino acids in the principal proteins of a large number of seeds, seed products, forage plants, roots, and animal tissues.

In addition to those given in the preceding classification, a number of other amino acids have been reported as products of protein hydrolysis, but general acceptance of their identity awaits further confirmation. All of the amino acids, with the exception of glycine, are optically active in nature. Most of them are soluble in water. Since amino acids have both amino groups and

<sup>1</sup> MITCHELL, H. H., and T. S. HAMILTON, The biochemistry of the amino acids, pp. 180-186, Chemical Catalog Company, Inc., New York, 1929.

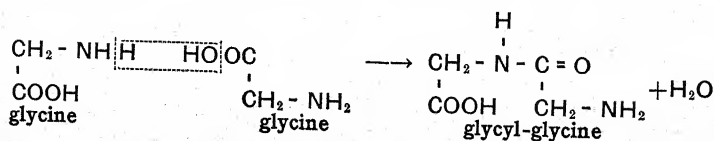
carboxyl groups, they are considered to be amphoteric electrolytes, reacting as acids in the presence of bases and as bases in the presence of acids. Different amino acids have different isoelectric points.

TABLE X.—PERCENTAGE OF AMINO-ACID CONSTITUENTS OF PROTEINS<sup>1</sup>

Proteins	Zein	Edes- tin	Casein	Gela- tin	Glia- din	Lactal- bumin
Glycocoll.....	0.0	3.8	0.45	25.5	0.0	0.37
Alanine.....	9.8	3.6	1.85	8.7	2.0	2.41
Valine.....	1.9	5.6	7.93	0.0	3.34	3.3
Leucine.....	25.0	8.1	9.7	7.1	6.62	14.03
Proline.....	9.0	4.1	8.7	19.7	13.22	3.76
Oxyproline.....	0.8	2.0	0.23	14.4		
Phenylalanine.....	7.6	3.09	3.88	1.4	2.35	1.25
Glutamic acid.....	31.3	19.2	21.77	5.8	43.66	12.89
Oxyglutamic acid.....	2.5	0.0	10.5	0.0	7.7	10.0
Aspartic acid.....	1.8	10.2	4.1	3.4	0.8	9.3
Serine.....	1.0	0.33	0.5	0.4	0.13	1.76
Tyrosine.....	5.9	4.5	6.55	0.01	3.04	1.95
Cystine.....	0.91	1.25	0.25	0.2	2.16	2.29
Methionine.....	2.35	2.39	3.50	0.97	2.03	2.62
Histidine.....	0.8	2.08	1.83	0.9	1.49	1.52
Arginine.....	1.8	15.8	3.85	8.2	2.91	3.00
Lysine.....	0.0	2.19	6.25	5.9	0.64	8.08
Tryptophane.....	0.17	1.5	2.2	0.0	1.09	2.69
Ammonia.....	3.64	2.28	1.95	0.4	5.22	1.31
Total.....	106.27	92.01	95.99	102.98	98.40	82.53

<sup>1</sup> Compiled by H. B. Vickery, Connecticut Agricultural Experimental Station, 1935, and reproduced by his courtesy.

**81. The Structure of the Protein Molecule.**—Our knowledge of the structure of proteins is due primarily to the work of Emil Fischer who devised methods for uniting amino acids through their amino and carboxyl groups, with the elimination of water. As an example, the union of two molecules of glycine to form the dipeptide, glycyl-glycine may be represented as follows:



Fischer succeeded in hooking together as many as 18 amino acids, and the complex synthetic polypeptides thus formed were found to have many properties in common with the proteins. Thus, on the basis of his and later work, it is considered that proteins are anhydrides of amino acids and that the principal linkage existing between the amino acids in the protein molecule is through the amino group of one acid and the carboxyl group of another. This type of union ( $-\text{NH} - \text{C}(=\text{O})$ ) is referred to as the peptide linkage.

It is assumed that the proteins occurring in nature are built up from their constituent amino acids, but we have no knowledge as to the exact process. In the case of plants, including the lower forms such as yeast and bacteria, nitrates and ammonium salts are used as the initial nitrogenous compounds for protein synthesis. In the case of animals, however, the constituent amino acids must be available, with the exception of some which can be synthesized in the body from simpler compounds, as is discussed later. The lower forms of animal life may have a greater ability to synthesize amino acids, but the evidence here is not conclusive.

**82. Properties of Proteins.**—Proteins can combine chemically with both acids and bases. This is explained on the basis that they are amphoteric substances by virtue of containing free amino and carboxyl groups. Each protein has its characteristic isoelectric point at which the tendencies to acidic and basic dissociations are equal, and, at this point, the protein is most readily precipitated by salt solutions and by alcohol. This property is taken advantage of in the separation and purification of proteins.

In addition to forming chemical combinations, proteins in solution also behave like colloids. They do not pass through the membranes or gels which are used as the criteria for separating crystalloids from colloids, and many workers hold the view that proteins can bind ions physically by adsorption as well as uniting with them chemically. After an exhaustive study Hoffman and Gortner<sup>1</sup> reached the conclusion that physical combinations take

<sup>1</sup> HOFFMAN, WALTER F., and ROSS AIKEN GORTNER, Physico-chemical studies on proteins. I. The protamines—their chemical composition in relation to acid and alkali binding, *Colloid Symposium Monograph*, 2, 209–358, 1925.

place outside the hydrogen-ion concentrations of pH 2.5 and 10.5 and that, inside of this range, chemical combinations occur. Others, however, adhere to the view of Loeb that the lack of diffusibility of the proteins is due merely to the size of the protein ion resulting from the high molecular weight of proteins and that their colloidal behavior is limited to this property. This concept of the nondiffusibility of the protein ion itself provides a basis for explaining the behavior of proteins according to Donnan's theory of membrane equilibrium (Sec. 10).

The different proteins vary as regards their solubility in water and various aqueous solutions, but none of them is soluble in the common fat solvents such as ethyl ether and petroleum ether. They can be precipitated from solution by a large variety of substances, including various neutral salts, such as sodium sulfate and magnesium sulfate. Large amounts of these salts are required to cause the proteins to precipitate, and the process is referred to as *salting out*. Salts of heavy metals are good precipitants and so are strong mineral acids. Phosphotungstic acid, trichloroacetic acid, and tannic acid are reagents commonly used in the laboratory for precipitating proteins.

The coagulation of protein probably involves a dehydration. It can be brought about by heat and by alcohol. In digestion it occurs as a result of enzymatic action.

**83. The Chemical Determination of Protein.**—The direct determination of the various proteins which are present in a feed or tissue is an impracticable procedure. Thus the chemist takes advantage of the fact that nitrogen occurs in the different proteins in a fairly constant percentage—16 per cent on the average (Sec. 76). He analyzes for nitrogen and multiplies the result by the factor 6.25 ( $100 \div 16$ ). The sample is digested with sulfuric acid and a catalyst until all the organic matter is destroyed, which procedure changes the nitrogen into the form of ammonium sulfate. The digest is distilled with sodium hydroxide and the ammonia is driven over into standard acid and titrated, whereby the nitrogen present in the sample is determined. While the average factor, 6.25, is applied to feeds in general, specific factors may be used in the case of products for which the protein and nitrogen relations are definitely known. For example, it has been found that the combined proteins of milk contain approximately 15.7 per cent of nitrogen on the average and hence the factor

6.38 is used. Specific factors are also employed for certain animal tissues. Jones<sup>1</sup> has published specific factors for 121 different proteins and foods.

The estimation of protein content from an analysis for nitrogen assumes that all of the nitrogen in the substance analyzed is in the form of protein. This is not strictly true for any feedstuff, and there are certain ones which contain a considerable amount of nitrogen in compounds other than protein. This fact was recognized by the early workers in animal nutrition and methods were accordingly proposed for determining the *true protein* as distinguished from the crude protein obtained by multiplying the total nitrogen by a factor. True protein as obtained by the *Stutzer method* is the insoluble residue resulting from the boiling of the feed with water and the addition of copper hydrate. The nitrogen is then determined in the residue and multiplied by 6.25. The method assumes that the nonprotein compounds are water soluble and that any protein so dissolved is precipitated by the boiling and by the copper hydroxide. The early workers recognized that the method was a conventional one and recent studies have emphasized its limitations. The distinction between crude and true protein, however, has largely lost its significance in so far as the protein values of feeds are concerned, for reasons which are detailed later (Sec. 88).

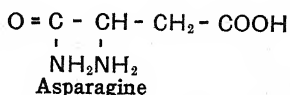
**84. Nonprotein-nitrogen Compounds.**—The nonprotein compounds occurring in feeds include: amides, amino acids, nitrogenous glucosides and fats, alkaloids, ammonium salts, and others. Of these, the amides and the amino acids are the only ones which occur to any considerable extent, and they are present in large amounts in only a few of the common feeds. They seem to be especially abundant where growth is rapid, and thus they may make up as much as one-third of the total nitrogen in pasture-grass and soiling crops. The developing seed is high in non-protein nitrogen at the start but low at maturity. A large part of the nitrogen in silage crops occurs in this form, due in part to their immaturity at harvest and in part to the fermentation processes which take place. Certain roots apparently have a considerable portion of their nitrogen stored in forms other than protein. Mature hays and the commonly fed concentrate

<sup>1</sup> JONES, D. BREESE, Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins, *U. S. Dept. Agr. Circ.* 183, 1931.

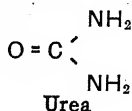
mixtures of seeds and their by-products contain relatively little nonprotein nitrogen.

In addition to the nonprotein nitrogen compounds which occur in feeds, there are a number which are important in nutrition, either as intermediary or end products of protein metabolism or as essential and active constituents of various tissues and secretions.

*Asparagine*, the monoamide of aspartic acid, was discovered in asparagus shoots in 1805, and, since that time, it has been found in many other plants. *Glutamine* is another amide of frequent occurrence in plants.

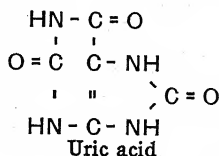


Many consider that mobilized protein is transported in the form of amides, but others ascribe this role to the amino acids. *Urea*, the diamide of carbonic acid, is the principal end product of nitrogen metabolism in mammals and in most fishes.



There are many different *nucleic acids* in nucleoproteins, distinguished by their hydrolytic products. The nucleic-acid molecule contains phosphoric acid, pentoses, purine bases, and pyrimidine bases. These bases are heterocyclic compounds containing nitrogen.

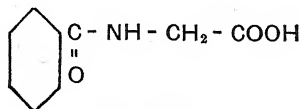
*Uric acid* is the principal end product of purine catabolism in man and of protein catabolism in birds and reptiles. Uric acid and urates are the principal nitrogenous excretory products in insects.



In mammals other than man and the monkey, the principal end product of purine catabolism is not uric acid but its oxidation product, *allantoine*,  $\text{C}_4\text{H}_6\text{N}_4\text{O}_3$ .



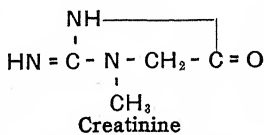
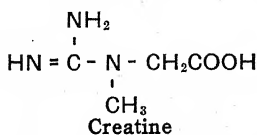
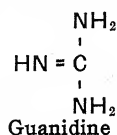
*Hippuric acid* occurs in considerable amounts in the urine of all herbivorous animals and normally in much lesser amounts in the urine of other animals including man. It is a combination of benzoic acid and the amino acid, glycocoll or glycine, and thus is named benzoyl-glycine.



Hippuric acid

It is classed as a detoxication product in that benzoic acid, which cannot be utilized by the body and is in fact harmful, is transformed, by pairing it with glycine, into a harmless product which is readily excreted. The rations of herbivorous animals normally contain much larger amounts of hippuric-acid precursors, such as benzoic acid and various related aromatic compounds, than is the case for Omnivora. In all species there are considerable variations in hippuric-acid excretion according to the nature of the feed.

Creatine, methylguanidine acetic acid, occurs widely in the animal body, especially in muscle. It is excreted as its anhydride, *creatinine*, which is thus a normal constituent of urine. Creatinine excretion is a measure of the basal nitrogen catabolism, as is discussed later (Sec. 226). The relation between these two compounds is indicated by their formulas:



Creatine occurs in muscle as an easily hydrolyzable phosphate, *phosphocreatine* or *phosphagen*, which is decomposed in muscle activity (Sec. 339).

*Glutathione*, is a tripeptide of cysteine, glutamic acid, and glycine, which plays a role in physiological oxidations.

#### AMINO ACIDS AND PROTEIN QUALITY

The recognition that the nitrogen present in the body had its origin in nitrogen compounds present in the food dates primarily

from the work of Magendie<sup>1</sup> published in 1816. After it became established that proteins were the nitrogen compounds essentially concerned, Magendie produced the first evidence that all proteins were not of equal value. In his famous "gelatin report" published in 1841, he showed that gelatin would not take the place of meat protein in the diet. Thirty-five years later Escher, in an experiment with dogs, reported that the deficiency of

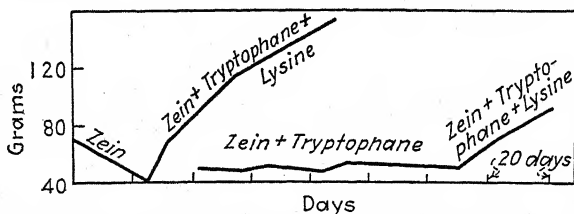


FIG. 7.—The effect on rat growth of supplementing the protein, zein, with tryptophane and lysine. (From Osborne and Mendel.)

gelatin could be made good by the addition of tyrosine. This finding was a forerunner of the modern concept of the essential role of amino acids in protein nutrition, a concept which did not become recognized until 40 years after the initial observation of Escher. In 1914 Osborne and Mendel<sup>2</sup> showed that certain proteins which resulted in nutritive failure when used alone were rendered satisfactory by the addition of missing amino acids. For example, as is illustrated in Fig. 7, on a diet otherwise adequate but containing zein as the sole protein, rats failed to grow and in fact lost weight. The addition of lysine and tryptophane, two amino acids not present in zein, resulted in the resumption of growth. The addition of tryptophane alone enabled the animal to maintain its weight but no growth occurred until lysine also was added. These fundamental results, quickly followed by many others, led to the realization that the value of a given protein in nutrition is governed by its amino-acid make-up,

<sup>1</sup> François Magendie (1783–1855), the great French physiologist, is recognized as the founder of the modern experimental method in animal feeding experiments. He employed diets of pure carbohydrates and fats to prove that food nitrogen is essential. These studies were published under the title: *Sur les propriétés nutritives des substances qui ne contiennent pas d'azote*, *Ann. chim. phys.*, 1st ser., 3, 66–77, 1816.

<sup>2</sup> OSBORNE, THOMAS B., and LAFAYETTE B. MENDEL, Amino acids in nutrition and growth, *J. Biol. Chem.*, 17, 325–349, 1914.

because the body is unable to synthesize many of the amino acids which are present in its proteins and thus the protein in the food must be of a nature which will supply them.

**85. Essential and Nonessential Amino Acids.**—Experiments have established a list of amino acids which the body cannot synthesize adequately and which are thus designed as *essential amino acids*. They have also established another list of acids classified as unessential, since the body can manufacture them. Some of this knowledge has been gained by experiments such as that illustrated in Fig. 7. The large advance in this field has come from the magnificent work of Rose and associates<sup>1</sup> of the University of Illinois, which has been in progress for several years. Using diets in which the sole source of nitrogen was supplied as amino acids, they have added or removed various of these acids and thus classified them as essential or nonessential. Their latest results, in part unpublished as this is written, are summarized in Table XI through the courtesy of Dr. Rose. It is noted that, of the 22 amino acids listed, 10 have been found essential and 12 nonessential.

Arginine represents a special case in that growth can take place in its absence but not at a normal rate. This means that this acid can be synthesized in the body but not sufficiently rapidly to meet the demands of normal growth. Dr. Rose has exactly defined an essential amino acid as one which cannot be synthesized at a sufficiently rapid rate to cause normal growth. The finding that cystine is not essential for growth is contrary to earlier accepted data. The early work on this acid was done before the other sulfur-containing amino acid, methionine, was discovered. Rose and associates have clearly proved that cystine can be omitted when methionine is present but that the reverse is

<sup>1</sup> WOMACK, MADELYN, and WILLIAM C. ROSE, Feeding experiments with mixtures of highly purified amino acids. VII. The dual nature of the "unknown growth essential," *J. Biol. Chem.*, **112**, 275-282, 1935.

MCCOY, RICHARD H., CURTIS E. MEYER, and WILLIAM C. ROSE, Feeding experiments with mixtures of highly purified amino acids. VIII. Isolation and identification of a new essential amino acid, *J. Biol. Chem.*, **112**, 283-302, 1935.

ROSE, WILLIAM C., KENNETH S. KEMMERER, MADELYN WOMACK, EDWIN T. MERTZ, J. KENNETH GUNTHER, RICHARD H. MCCOY, and CURTIS E. MEYER, The present status of the amino acids in nutrition, *J. Biol. Chem. Proc.*, **114**, LXXXV, 1936.

not true, and their studies have explained why the earlier workers obtained a growth response with cystine.

While additional amino acids may be discovered in plant or animal proteins, it is clear from these Illinois investigations that all of the essential ones are known, at least in so far as the growth of rats is concerned. Although the experiments have not covered the entire growth period, it is very improbable that prolonged

TABLE XI.—CLASSIFICATION OF THE AMINO ACIDS WITH RESPECT TO THEIR GROWTH EFFECTS<sup>1</sup>

<i>Essential</i>	<i>Nonessential</i>
Lysine	Glycine
Tryptophane	Alanine
Histidine	Serine
Leucine	Norleucine
Isoleucine	Aspartic acid
Phenylalanine .	Citrulline
Threonine	Tyrosine
Methionine	Proline
Valine	Hydroxyproline
Arginine	Glutamic acid
	Hydroxyglutamic acid
	Cystine

<sup>1</sup> As reported by W. C. Rose at the Annual Meeting of the Federation of American Societies for Experimental Biology, Memphis, Tenn., April, 1937.

observations would disclose requirements beyond those noted during the period of most rapid growth. It must be recognized, however, that the requirements for other body functions, such as lactation, egg production, and wool production, may be somewhat different. Quantitative differences, at least, are to be expected.

The studies of the Illinois workers which have resulted in the data presented in Table XI have involved a tremendous amount of work in the preparation of pure amino acids, as well as hundreds of carefully controlled feeding experiments. To duplicate the work for a farm animal would be an almost impossible task, and, certainly, the cost would be prohibitive until cheap sources of amino acids become available. While it must be recognized that species differences in ability to synthesize amino acids may exist, there is ample evidence that the concept of essential and non-essential acids applies in the case of farm animals and that data obtained with rats find application in the nutrition of the larger species.

It is noted that Table XI includes one amino acid not listed in the previous classification (Sec. 79), citrulline,  $\delta$ -carbamido- $\alpha$ -amino-valeric acid. The table does not include thyroxine and iodogorgoic acid, which are derivatives of the nonessential amino acids, alanine and tyrosine, respectively.

**86. Essential Amino Acids and Protein Quality.**—This discovery that many of the amino acids composing body proteins must be supplied as such by food protein explains why different foods and rations of the same protein content have different protein values in nutrition, *i.e.*, they differ in *protein quality*. This may be illustrated as follows:

Let, A, B, C, D represent essential amino acids,

$A_3B_6C_4D$  represent a body protein, and

$A_3B_6C_4$  (1),  $A_2B_6C_2D$  (2) and  $A_3B_6C_4D_2$  (3) represent food proteins

Food protein 1 is entirely useless to build the body protein because it does not supply the essential amino acid D, present in the body protein. Food protein 2 supplies all the amino acids, but it would require two molecules of it to furnish enough of the amino acid C to form the body protein, and the following residue would be left unused:  $AB_6D$ . Thus approximately one-third of this food protein would be of no value for building body protein. In contrast one molecule of food protein 3 would form one molecule of the body protein with only one amino acid, D, left over. Thus it is a much more efficient source than protein 2. It ranks higher in *protein quality* or *biological value*.

In actual feeding practice, no ration ever contains only one protein, but consists of a mixture of many. No ration is ever likely to be entirely lacking in an essential amino acid, but different rations differ widely in the quantitative relations of their amino acids. Thus the ration which has the highest protein quality is the one which supplies all the amino acids needed in proportions most nearly like those in which they exist in the protein to be formed (growth tissue, milk, eggs, wool). Such a ration will meet the protein needs of the body with a minimum intake of food protein. On the other hand, certain rations may be so deficient in one or more essential amino acids that they will not provide adequate protein nutrition no matter how much is fed. The amino acids which remain unused in the formation of body protein are not wasted as food because they can serve as a source

of energy, but they are wasted in so far as the specific function of protein is concerned.

Since the body contains many different proteins having somewhat different amino-acid relations, the usefulness of the food protein depends in part on the purpose for which it is fed. The amino-acid requirements for maintenance may thus be somewhat different from those of growth and also different from those for milk production.

**87. The Determination of Amino Acids in Feeds.**—Since feeds differ in protein value according to the kinds and amounts of essential amino acids present, it obviously would be highly useful to have a simple and accurate method of analyzing feeds for amino acids, to provide data for computing rations. This fact was early recognized and in 1915 papers by Grindley and associates<sup>1</sup> and by Nollau<sup>2</sup> appeared giving results obtained by the Van Slyke method previously applied to pure proteins. Other publications, particularly by Grindley and associates, followed and the more reliable results for some 30 feeds have been tabulated by Mitchell and Hamilton.<sup>3</sup> Recently some additional values have been reported by Morris.<sup>4</sup>

There is considerable lack of agreement among some of the published data. It has been mentioned that the quantitative determination of amino acids in isolated proteins is difficult and does not give exact results. The difficulties are multiplied many times when one deals with a mixture of proteins in complex association with the large number of other substances which occur in plant products. The procedure can be regarded only as approximate at best. Further, only a few of the amino acids now classed as essential are accounted for in the publications cited.

<sup>1</sup> GRINDLEY, H. S., W. E. JOSEPH, and M. E. SLATER, The quantitative determination of the amino acids of feeding-stuffs by the Van Slyke method, *J. Am. Chem. Soc.*, **37**, 1778-1781, 1915; GRINDLEY, H. S., and M. E. SLATER, The quantitative determination of the amino acids of feedingstuffs by the Van Slyke method, *ibid.*, **37**, 2762-2769, 1915.

<sup>2</sup> NOLLAU, E. H., The amino-acid content of certain commercial feeding-stuffs and other sources of protein, *J. Biol. Chem.*, **21**, 611-614, 1915.

<sup>3</sup> MITCHELL and HAMILTON, *op. cit.*, p. 190.

<sup>4</sup> MORRIS, SAMUEL, The nutritive value of proteins for milk production. III. The determination of the amino acids of various feeds, *J. Dairy Research*, **5**, 108-112, 1934.

In view of these various considerations, as well as the fact that we have little direct knowledge as to what amino acids are actually essential for the various functions in farm animals, it is clear that for the present at least we must look to other methods for arriving at the biological value of the protein feeds. Such methods are discussed later (Sec. 254).

**88. Crude vs. True Protein.**—The recognition that the value of feed for protein nutrition is determined primarily by its amino-acid content has caused the distinction between crude and true protein (Sec. 83) to lose the significance formerly attributed to it. According to the concept of true protein, any uncombined amino acids present in a feed are eliminated from consideration; yet they are just as valuable as though actually a part of a protein molecule. Hart and Bentley<sup>1</sup> found for various seeds, hay, and green forage that the nitrogen classed as nonprotein by the Stutzer procedure was largely comprised of free amino acids and peptides. Certainly a determination of protein which does not include these acids and peptides is not a true measure of the value of a feed in protein nutrition. Further, true protein itself does not have the certain and fixed nutritive value originally conceived for it. Its value is dependent upon the kinds and amounts of amino acids contained in it. Finally there is evidence, as is referred to later (Sec. 90), that, in addition to the amino acids, there are certain other nonprotein nitrogen compounds eliminated in the determination of true protein, which are not entirely useless for meeting the protein needs of the body.

In view of all these considerations, a distinction between crude and true protein of feeds, particularly as conceived of by the Stutzer method, seems no longer worth while. In this book, unless otherwise specifically stated, the term, protein, is used without qualifying adjective to express the value obtained by multiplying the total nitrogen by 6.25 (or some other stated factor). It should be borne in mind that the value so expressed includes other nitrogen compounds besides protein and that a more exact measure of the value of feed nitrogen for protein nutrition must at present be a biological rather than a chemical one.

<sup>1</sup> HART, E. B., and W. H. BENTLEY, The character of the water-soluble nitrogen of some common feedingstuffs, *J. Biol. Chem.*, **22**, 477-483, 1915.

## PROTEIN METABOLISM

**89. Products of Protein Digestion.**—As a result of the action of the various proteolytic enzymes secreted into the stomach and intestines, the food proteins are hydrolyzed through various intermediary stages to their constituent amino acids. The latter are thus the principal end products of protein digestion, but a small amount of ammonia may also be produced as a result of the splitting off of free amino groups from the protein molecule, or from some of its intermediary hydrolytic products, or from amides.

**90. Bacterial Action in the Digestive Tract.**—In the discussion of carbohydrate metabolism, it was pointed out that bacteria and other microorganisms play a large role in the breakdown of complex carbohydrates in the digestive tract, especially in the Herbivora. This bacterial growth has a significance as regards protein metabolism as well. As the bacteria multiply, they synthesize protein to construct their own bodies, obtaining the raw material from the ingested food. For this purpose they can utilize amides, ammonium salts, and even nitrates, as well as protein itself. Bacterial protein so formed in the rumen may be digested later in the stomach and intestine. To the extent that this occurs, there may be available for absorption a different mixture of amino acids than would have resulted from the digestion of the food protein alone, and, in so far as the bacterial protein is formed from the simpler nitrogen compounds which the animal body cannot utilize, a distinct gain in amino acids available to the body may result.

The concept that microorganisms play a useful role in protein metabolism was put forward long before the specific importance of amino acids was appreciated. From a study of cellulose digestion Zuntz,<sup>1</sup> in 1891, expressed the view that rumen bacteria use by preference amides, amino acids, and ammonium salts instead of protein. Other studies led to the belief that the protein

<sup>1</sup> Nathan Zuntz (1847-1920) was a pioneer in the field of basal metabolism and in respiration studies with farm animals. He developed the first portable respiration apparatus. Trained as a physician, he early forsook medicine to become a teacher and investigator in physiology, first at Bonn and later at Berlin. He devoted himself particularly to work with farm animals and to basic problems related to their nutrition. His publications, numbering over 400, deal with a wide variety of physiological problems.



supplied by a given ration was augmented as a result of the formation of protein in the bodies of bacteria and protozoa which were later digested. These early observations were followed by many experiments indicating that the protein requirements of animals, especially Herbivora, could be met in part by such nonprotein nitrogen compounds as asparagine, urea, and even ammonium salts, and these findings have frequently been explained on the basis that microorganisms intervene to transform these simple compounds into protein which is later digested and thus serves the body.

Unfortunately few of these experiments have been conducted in a sufficiently critical way to make their results conclusive. In many cases the nonprotein compounds have been substituted in a ration in which the protein content was apparently unnecessarily high for the body function being supported, while in others critical proof that the protein nutrition was adequate after the substitution was not furnished. Further, by no means all of the experiments are in agreement. In reviewing the experiments up to 1925, Scharrer and Strobel<sup>1</sup> came to the conclusion that further studies were needed to provide certain evidence of the practical importance of this role of microorganisms in protein metabolism. In a more recent review, Terroine<sup>2</sup> draws the conclusion that certain amides are useful for meeting the needs of maintenance, growth, and milk production in polygastric animals and that they can partially cover the needs for maintenance in animals with one stomach. He feels that there is also positive evidence for the usefulness of ammonium salts, but that the evidence is very questionable in the case of urea. He points out, however, that all of these nonprotein compounds are less useful than protein itself.

While bacterial action is generally considered as the principal explanation for the usefulness of amides and ammonium salts in protein nutrition, the additional explanation is frequently offered that ammonia split off from these compounds may be useful for combating deamination by mass action and that it may also

<sup>1</sup> SCHARRER, K., and A. STROBEL, Das Problem des Eiweissersatzes durch Ammoniumsalze und Amidostoffe bei der Tierernährung, *Z. angew. Chem.* **38**, 601-609, 1925.

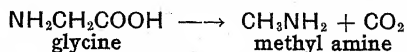
<sup>2</sup> TERROINE, ÉMILE F., Le métabolisme de l'azote, pp. 384-452, Les Presses Universitaires de France, Paris, 1933.

serve by uniting with organic acids from carbohydrates to form nonessential amino acids (Sec. 85).

Since the intervention of bacteria may alter the proportion of the different amino acids available for absorption, the possibility that protein quality may be thus altered must be recognized. This is offered as an explanation of the apparently lesser importance of the protein-quality concept for Herbivora than for Omnivora as is referred to later (Sec. 257). The importance of the right proportion of amino acids in the feed as ingested may become less thereby.

That the formation of protein as a result of the growth of bacteria and protozoa is of sufficient magnitude to be of quantitative significance is indicated by the work of Schwarz.<sup>1</sup> In a study of the nitrogen distribution of the rumen contents, this investigator found that over 30 per cent of it was present in the bodies of microorganisms. The explanation that bacteria may convert some of the nonprotein nitrogen compounds of a ration into protein which is later released as amino acids constitutes a further reason why a distinction between crude and true protein is of no significance (Sec. 88).

**91. Putrefaction.**—In addition to the previously described roles of microorganisms in the digestive tract, which are evidently advantageous from a nutrition standpoint, bacteria are also responsible for putrefactive changes which are clearly undesirable and even harmful. Amino acids or partially digested proteins are broken down by certain bacteria primarily in the large intestine, with the formation of amines, phenols and other alcohols, various organic acids, and other compounds. Amino acids are changed to amines by decarboxylation:



Thus histidine is changed to histamine, and tyrosine to tyramine. Other putrefactive changes are illustrated by the formation of phenol from phenylalanine and tyrosine, indole from tryptophane, and methyl mercaptan and hydrogen sulfide from cystine.

To the extent that these changes occur, nutritionally useful nitrogen compounds are converted into useless ones, many of

<sup>1</sup> SCHWARZ, CARL, Die ernährungsphysiologische Bedeutung der Mikroorganismen in den Vormägen der Wiederkäuer, *Biochem. Z.*, **156**, 130–137, 1925.

which may be definitely harmful. To protect itself against such harmful effects, as well as against certain toxic substances in the food, the body has specific defense mechanisms, referred to as *detoxications*, which are generally considered to be centered in the liver. These mechanisms involve the transformation of the toxic substance, frequently by joining it with another compound, to form a harmless substance which can be excreted. In this way several of the putrefactive products are conjugated with either sulfuric acid or glucuronic acid. The pairing of benzoic acid with glycine to form hippuric acid has been mentioned.

Putrefaction is increased by a high-protein diet, by *constipation*, and by conditions in the intestine which are especially favorable to the development of the organisms in question. Excessive putrefaction, which results in the entrance of toxic products into the general circulation, reveals itself in the human in bad breath, loss of vigor, skin eruptions, and other symptoms. In farm animals it may be responsible for lack of appetite, rough coat, and general decline in condition. While these harmful effects unquestionably occur, they are frequently overemphasized. Ordinarily the defense mechanisms are adequate to protect the body against them.

**92. Fecal Nitrogen.**—The nitrogenous compounds excreted in the feces consist in part of undigested or unabsorbed food nitrogen and in part of another fraction, called *metabolic nitrogen*. This metabolic fraction comprises substances originating in the body, such as residues of the bile and other digestive juices, epithelial cells abraded from the alimentary tract by the food passing through it, and bacterial residues. Strictly speaking, however, the nitrogen in bacterial residues must be considered to have come originally, in part at least, from the food. The existence of this fecal metabolic nitrogen, as distinguished from undigested nitrogen, is shown by the fact that the feces excreted on a nitrogen-free diet always contain nitrogen compounds. The reason for making the distinction is that the two fractions have different origins and that the distinction is made use of in measuring the biological value of proteins in nutrition (Sec. 255).

While the amount of the undigested fraction is determined by the digestibility of the nitrogenous portion of the ration, the size of the metabolic fraction is independent of this factor and is governed in part by the total amount of dry matter consumed and

in part by body size. That metabolic nitrogen output increases with the level of food intake is easily understandable from the fact that the higher the intake, the greater the secretion of digestive juices and the greater the wear and tear on the lining of the tract. The output per unit of dry-matter intake is apparently increased as the proportion of roughage increases in the diet, but the protein level has practically no influence. Data obtained by Mitchell indicate that on rations containing minimum amounts of roughage the output of metabolic nitrogen is approximately 0.2 g. per 100 g. of dry matter consumed.

The influence of body size is shown by observations that at the same level of food intake the larger animal excretes more metabolic nitrogen. This effect is explainable on the basis that some of the nitrogenous constituents of the bile, and possibly of other digestive secretions, represent true excretions of body metabolism unrelated to food intake. One would expect these excretions to be proportional to body size. The relative influence of food intake and of body size upon the amount of metabolic nitrogen has been critically studied by Schneider<sup>1</sup> working in Mitchell's laboratory, and this investigator presents an extensive review of the subject. While the preponderance of the evidence indicates that food intake has the predominant influence, some workers have come to the opposite conclusion.

It is evident that, at a given level of food intake, the higher the digestibility of its protein, the larger is the proportion of the total fecal nitrogen which is represented by the metabolic fraction. In the case of a man eating a highly digestible diet, the fecal nitrogen is principally metabolic. At the other extreme, the metabolic nitrogen may be only a small fraction of the total fecal output in the case of Herbivora consuming a ration of low protein digestibility. To the extent that the metabolic nitrogen is a function of total food intake, lowering the proportion of protein in the ration results in a decrease in the undigested nitrogen relative to the total fecal output.

Various methods have been used for separating the metabolic from the undigested nitrogen in the feces. The one most commonly employed involves the digestion of the feces by a pepsin

<sup>1</sup> SCHNEIDER, BURCH HART, The relationship of the metabolic nitrogen of the faeces to body weight and to food intake for rats, *Biochem. J.*, **28**, 360-364, 1934.

solution acidified with hydrochloric acid. Sometimes a subsequent digestion with trypsin is used. The basis of the method is that the procedure will digest the nitrogenous compounds of metabolic origin but not break down the undigested fraction since the latter has previously resisted a similar action in the body. The method is open to criticism from several points of view and various studies have shown it to be inaccurate. The output of metabolic nitrogen can be accurately studied with a nitrogen-free ration or with rations containing small amounts of proteins which are known to be practically 100 per cent digested. Figures so obtained for the metabolic nitrogen per unit of dry-matter intake are used to calculate the metabolic fraction produced from rations which result in the excretion of undigested nitrogen as well.

**93. Apparent and True Digestibility of Protein.**—The recognition that the fecal nitrogen consisted in part of compounds other than those coming from the food early led to the proposal to determine the true digestibility of protein by considering only the undigested fraction, as distinguished from apparent digestibility which was based on the total output. The previous discussion has indicated that there is no accurate method for quantitatively determining the two fractions. While such a distinction is desirable in certain experimental work, it does not have any important significance in feeding practice. The excreted metabolic nitrogen represents a loss which must be taken account of and assessed against some body process. Although it is independent of the nitrogen component of the food, it is related to the food intake as a whole and it is a loss which occurs in the course of the digestion of this food. It is more appropriately assessed against digestion than against any other body function. Thus the figures for the digestibility of protein commonly determined and employed represent apparent digestibility, though generally spoken of without this qualifying term.

**94. The Disposal of Absorbed Amino Acids.**—The nitrogenous digestion products enter the blood stream mainly, at least, as amino acids. Small amounts of ammonia and of the simpler peptides are also absorbable. Occasionally, traces of proteins, as such, are absorbed, in which case they act as foreign bodies. The absorption of amino acids causes their level in the blood to rise temporarily, but they are rapidly removed from the circulating fluid by the various tissues of the body and they are held for a

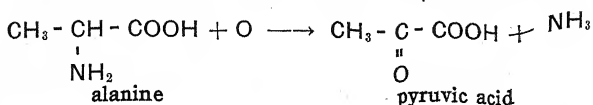
time in the tissues in a very loosely combined or in a free state. Subsequently they are disposed of in the following ways:

1. They may be synthesized into tissue proteins and other nitrogen-containing tissue constituents. Such a synthesis includes the formation of the protein and other nitrogenous compounds of secretions, notably of milk. It also involves the replacement of tissues used up in the "wear and tear" of body processes.

2. They may be deaminized and the nonnitrogenous residue used currently as a source of energy, or this residue may be converted into glycogen, or perhaps even into fat, to be held as reserve for future energy needs.

The mechanism of protein synthesis from amino acids is unknown. In vitro, it can be accomplished by the same enzymes which hydrolyze protein, and thus the synthesis is sometimes considered as a reverse phase of proteolysis. But the conditions under which it can be made to take place in vitro are vastly different from those which are met in the body, and thus the actual body process is far from being explained. In the adult animal which is producing no special nitrogenous product, such as milk, eggs, wool, or even a fetus, the synthesis of amino acids into protein is limited almost entirely to the requirements for the replacement of worn-out tissues: for, differing from the case for carbohydrates and fat, protein, as such, cannot be stored by the adult organism except to a very limited extent. Thus any excess of amino acids undergoes deaminization. Not only is this true for the absorbed amino acids as a whole, but it is true also for specific ones present in an excess relative to others which are essential for protein synthesis (Sec. 86). Thus, in rations containing protein of low biological value, an extensive deaminization of absorbed amino acids inevitably occurs, irrespective of whether the needs for protein synthesis are completely met. It is an open and important question as to whether deaminization starts to any appreciable extent before tissue needs are satisfied.

This deaminization occurs in the liver and kidneys and perhaps elsewhere. It involves the splitting off of ammonia, either by oxidation or by hydrolysis, as is indicated by the following equation for the oxidative procedure:



95. "Deposit Protein."—The previous statement that the adult organism cannot store protein requires qualification. During a period of inadequate protein nutrition, protoplasm may be broken down to meet certain other nitrogen needs of the body, and it may also be broken down to supply energy after a prolonged period of inadequate food intake. With the restoration of an adequate diet, protein will be retained in the adult body until the losses are made good. Further, exercise can result in increased muscle size in the adult, and this must mean some increase in protein content. In addition to these special cases of protein retention, it is recognized that a liberal protein intake regularly tends to result in a small increase in the body which is variously referred to as *deposit protein*, *labile protein*, *circulating protein*, and by other terms.

It is agreed that the nitrogen so retained is not built into protoplasm itself but that it exists in the fluids of the tissues, notably in the liver. Some refer to it as an unorganized form of protein, while others consider that it exists as amino acids. Mitchell describes it as "the very flexible reservoir of nitrogenous material that is increased with each increase in protein intake, and that is slowly depleted without detriment to the animal on lowering the level of protein nutrition." The proof of its existence rests upon nitrogen-balance studies (Sec. 194). Despite lack of agreement as to the nature of this retained nitrogen and the extensiveness of its retention, its occurrence is generally accepted, and it helps explain the results of certain protein metabolism studies, as is referred to later. This "deposit protein," however, can hardly be considered a reserve in the same sense that this term is used for fat deposits or for the glycogen in the liver and muscles, and quantitatively it is very small in amount.

96. Protein Catabolism.—When the tissues break down, the protein molecules are apparently hydrolyzed into their constituent amino acids by a process analogous to digestion. These amino acids next undergo deaminization, presumably in the same way as do some of the absorbed amino acids. The ammonia is converted into urea, mainly in the liver, and then excreted in the



urine. The nonnitrogenous residue serves as a source of energy, thus being broken down to carbon dioxide and water and so excreted. In addition to urea, the urine contains several nitrogenous catabolic products such as uric acid, ammonium salts, creatinine, hippuric acid, and others. These have been described previously (Sec. 84).

**97. Endogenous and Exogenous Catabolism.**—In 1905 Folin<sup>1</sup> put forth the theory that there are two forms of protein catabolism, essentially independent and quite different from each other; a variable one which he called *exogenous*, characterized by the excretion of urea and ammonium salts, and another tending to be constant, the *endogenous*, represented largely by the excretion of creatinine, neutral sulfur, and, to a lesser extent, uric acid and ethereal sulfates. According to this theory the endogenous catabolism represents metabolic processes which are the essential characteristic of living cells, exemplified by the nitrogen excretion on a nitrogen-free, otherwise adequate, diet. It reflects metabolic processes which are essential to life, and the end products thus excreted tend to be constant, unaffected by the character or amount of the food protein. The endogenous nitrogen excreted represents the loss which must be made good by dietary protein in order to maintain the integrity of the nitrogenous tissues of the body. The exogenous catabolism represents the breakdown of absorbed dietary nitrogen compounds which are not synthesized into body protein. Clearly, it may be highly variable according to the level of protein intake, the needs of the body for protein for other functions besides maintenance, and the suitability (biological value) of the absorbed nitrogen for meeting these needs.

The concept of an endogenous catabolism of nitrogen independent of the total protein catabolism is important for the student of nutrition, because it is utilized in arriving at the maintenance requirement (Sec. 234) and because it is taken account of in a common method of determining the biological value of proteins (Sec. 255). The Folin theory is difficult to prove or disprove experimentally. Many experiments have led to the conclusion that this endogenous catabolism, on a diet adequate in energy, does not necessarily involve the destruction of organized-tissue protein, though this undoubtedly occurs if the energy intake

<sup>1</sup> FOLIN, OTTO, A theory of protein metabolism, *Am. J. Physiol.*, **13**, 117-138, 1905.



is insufficient to meet the needs of the vital body processes. An excellent, detailed discussion of exogenous and endogenous metabolism with particular reference to the Folin theory has been presented by Mitchell and Hamilton.<sup>1</sup> The question of the validity of the theory in so far as it forms a basis of methods of studying nutritive requirements is considered later (Chap. XII).

The term endogenous, as employed by Folin and as generally used at the present time, refers exclusively to urinary nitrogen, but some workers employ the term to include the metabolic nitrogen of the feces also or refer to the latter as *fecal endogenous nitrogen*. This fecal nitrogen is endogenous in the sense that it is independent of protein intake, but it is a different kind of a waste from that represented by the urinary nitrogen of a nitrogen-free diet, with the exception of the amounts of catabolic products which are excreted into the gut in the bile and perhaps other channels. It has been explained that, from the standpoint of animal nutrition, it seems preferable to assess the losses of fecal, metabolic nitrogen against digestion. The term endogenous, at least as used without a qualifying adjective, should be considered to mean urinary nitrogen only.

**98. Protein-sparing Action.**—When carbohydrate is fed to a fasting animal the protein catabolism is decreased as shown by a smaller output of nitrogen in the urine. This is readily explainable on the basis that during fasting some of the body protein is being catabolized to furnish energy and that this becomes unnecessary with the intake of energy-yielding food. But many experiments have shown that carbohydrates exert a protein-sparing action even when the fuel supply is adequate. Whatever the protein level of an energy-adequate diet, the addition of carbohydrate tends to decrease the excretion of urinary nitrogen and thus to conserve or increase the body protein, though the minimum endogenous nitrogen on a protein-free, energy-adequate diet may not be reduced further by the addition of digestible carbohydrate. No generally accepted explanation of this protein-sparing action is available. It has been suggested that the increased concentration of easily oxidizable glucose which thus results in the tissues, tends to lessen the oxidation of amino acids. Another suggested explanation is that acids such as pyruvic and lactic from glucose metabolism unite with ammonia from protein

<sup>1</sup> MITCHELL and HAMILTON, *op. cit.*, Chap. IX.

catabolism to reform certain of the nonessential amino acids. Thus nitrogen which would have been excreted is restored to a form which can be utilized again. This protein-sparing action improves the efficiency of protein nutrition, but taking advantage of it does not necessarily mean optimum protein nutrition and it may be wasteful of energy.

**99. Minimum and Optimum Protein Intake.**—The question as to whether a level of protein in excess of the minimum required to meet the protein needs of the body is advantageous or disadvantageous has been long debated in the field of human nutrition, and it has implications for animals also. Under the influence of Liebig,<sup>1</sup> who erroneously believed that protein was broken down to furnish the energy for muscular work, the importance of large intakes of proteins was greatly overemphasized for many years following the middle of the last century. Gradually, as a result of research, the pendulum swung to the other extreme marked by the publication in 1904 by Chittenden<sup>2</sup> of experiments supporting the view that minimum intakes favored health and bodily vigor. Chittenden's views were by no means universally accepted, and, today, most authorities favor intakes in excess of what may be considered the minimum requirements.

We have seen that liberal protein intake tends to cause a high level of "deposit protein" in the tissues. The view is held by many that the presence of this excess supply has a beneficial physiological effect. This theory of optimum protein nutrition has been particularly applied to the dairy cow on the basis of experiments suggesting that raising the protein level stimulates the mammary glands. But there are many negative experiments also. Critical proof that the feeding of protein in excess of that

<sup>1</sup> Justus von Liebig (1803–1873) was the foremost organic chemist of his time and is frequently spoken of as the founder of agricultural chemistry. He was the father of the modern methods of organic analysis, and with him began the accumulation of knowledge regarding the composition of foods, tissues, feces and urine not available to earlier nutrition workers. He wrote several books dealing with the relations of organic chemistry to agriculture and to animal economy which are well worth reading by the modern student.

<sup>2</sup> Russell H. Chittenden (1856– ) served for 40 years as professor of physiological chemistry at Yale University where he made many outstanding contributions to the modern science of nutrition and inspired a host of students who have continued his work. An account of his protein studies is given in his book "Physiological Economy in Nutrition," Frederick A. Stokes Company, New York, 1904.

covering all requirements of the body will, by its presence or catabolism, stimulate production must be considered to be lacking.

On the other side, it is recognized that protein in excess of what the body can use tends to be wasted in so far as its specific function as protein is concerned, since it cannot be stored in any but very limited amounts but must be catabolized. Further, there are those who feel that excess protein is definitely harmful, stating that its catabolism and the excretion of urea place an unnecessary and undesirable burden on the body, particularly on the kidneys. There are many experiments showing the harmful effect of the excessive feeding of specific amino acids. On the other hand, there is ample evidence that the body can metabolize rather high levels of proteins in complete diets for long periods without deleterious effects on production or on the body itself.

Some of the differences of opinion relative to the optimum level of protein intake have doubtless arisen from failure to recognize the bearing of variations in protein quality. The minimum intake which proves adequate for a specific combination of proteins will not be either adequate or optimum for a combination of lower biological value. It seems clear that, at least during the growth period, the body can function normally over a rather wide range of protein intake above the minimum, except where certain diseases are present. Since marked differences in quality exist among commonly fed rations, at least for certain species, the intake in practice should be more liberal than the experimentally determined minimum with a specific combination. There is also evidence (Sec. 218) that a certain level is desirable from the standpoint of the most effective use of the ration as a whole. But that there are stimulating or other definite, though intangible, beneficial effects from a *luxus consumption* appears very doubtful.

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## CHAPTER VII

### THE INORGANIC ELEMENTS AND THEIR METABOLISM

The discussion in Chap. II has indicated that the body contains a large number of mineral elements which occur both in combination with each other and in combination with the organic constituents. While the physiological importance of some of them was early recognized, our specific knowledge of their nutritional significance is due primarily to research carried out during the last 25 years. Thus minerals form an important part of what is spoken of as the newer knowledge of nutrition.

**100. Essential Mineral Elements.**—Today we recognize that the following mineral elements perform essential functions in the body and thus must be present in the food: calcium, phosphorus, sodium, potassium, chlorine, magnesium, iron, sulfur, iodine, manganese, copper, and zinc. The proof that each of these elements is essential rests upon experiments with one or more species. All elements have not been tested with all species, but it is highly probable that there is no exception to the need for all of them by all higher animals. There are many other elements which are regularly found in the animal body. Of these, cobalt appears to be essential according to very recent work, but no necessary function has been reported for silicon, fluorine, boron, bromine, aluminum, arsenic, or nickel. Some of these elements may perform essential functions or they may be retained in small amounts in the body merely because they are present in the food.

The proof that a given element is essential depends upon feeding a ration so low in that element that structural or functional injury results. It is not possible to prepare any ration absolutely free from any mineral element, and thus proof of the need of those which may be required only in traces is difficult or impossible to establish. This explains why so many remain in the doubtful column. For example, while fluorine is a regular constituent of the bones and teeth of all animals, diets for rats containing as low

a level as one part in ten million have not been benefited in any observable way by fluorine additions. This does not prove, however, that fluorine is entirely unessential, for the trace which still remained in the diets may be all that the body needs. But the finding does suggest that any need for fluorine is of no practical importance, since it is very improbable that any normal diet fed for any extended period would contain as small an amount as mentioned above. This situation also illustrates a fact which greatly simplifies mineral nutrition in practice; *viz.*, that while at least twelve elements are absolutely essential, most of them are supplied so abundantly in the commonly fed rations that no thought need be given to them.

**101. Ash.**—The mineral elements as a group are determined in a feed or animal tissue by burning off the organic matter and weighing the residue, which is called ash. Such a determination tells nothing about the specific elements present and the ash may include carbon from organic matter as carbonate when base-forming minerals are in excess. The determination is used in the conventional feed analysis to provide a figure which can be added in with others to arrive at the nitrogen-free extract by difference (Sec. 39). The ash may be used as a starting point for the determination of the percentages of the specific elements present, the information in which we are interested from the standpoint of mineral nutrition. But it must be remembered that an analysis of the ash tells us nothing as to the combination in which a given mineral occurs either in a body tissue or in a feed. When the organic matter is oxidized the minerals present in organic combination are changed to an inorganic form. Many of the minerals in the body function primarily as specific organic and inorganic combinations, and, in the case of the food also, the combination is important in so far as the usefulness of certain elements are concerned. For example, the primary need for sulfur in the food is as a constituent of the amino acids, cystine and methionine. No information as to the amount of the element so combined is furnished by determining the sulfur content of the ash of the ration. Thus the nutrition chemist must resort to special methods which give him specific information as to the forms in which certain mineral elements occur in the body tissues and in foods, rather than relying on a determination of the ash and its ingredients.

**102. General Functions of Mineral Elements.**—The essential elements serve the body in many different ways. As constituents of the bones and teeth, they give rigidity and strength to the skeletal structures. They are also constituents of the organic compounds, such as protein and lipids, which make up the muscles, organs, blood cells, and other soft tissues of the body. Further, they serve a variety of functions as soluble salts in the blood and other body fluids. Here they are concerned in the maintenance of osmotic relations and acid-base equilibrium and exert characteristic effects on the irritability of muscles and nerves. Many of their vital functions are due to an interrelationship of ions which finds expression in the terms "antagonistic action" and "balanced solution." For example, if the normal level of sodium chloride (0.7 per cent) in the blood is increased by injection, the kidneys let blood and sugar pass into the urine. But the injection of sodium chloride plus salts of potassium, magnesium, and calcium does not upset kidney function. As another example, a certain balance between calcium, sodium, and potassium in the fluid which bathes the heart muscle is essential for the normal relaxation and contraction which constitute its beating. In addition to these general functions in which several minerals may take part, each one has various specific roles as is discussed later.

#### CALCIUM AND PHOSPHORUS

Over 70 per cent of the ash of the body consists of calcium and phosphorus. These two elements are discussed together because they are closely associated with each other in metabolism. They occur in the body combined with each other for the most part, and an inadequate supply of either in the diet limits the nutritive value of both. As early as 1842, it became recognized through the work of Chossat<sup>1</sup> with pigeons that poor bone developed on a diet low in calcium. When fed wheat alone, the birds died after 10 months, and, on post mortem, the bones were found very much depleted. Calcium carbonate prevented the trouble. Chossat used chickens, rabbits, frogs, eels, lizards, and turtles in later studies. During the next 20 years, studies in both France and Germany showed that skeletal development in various species of

<sup>1</sup> CHOSSAT, M., Note sur le système osseux, *Compt. rend. acad. sci.*, 14, 451-454, 1842.



farm animals was dependent upon the supply of calcium and phosphorus in the ration and that the deficiencies could be corrected by feeding bone meal and other sources of the minerals. Early in the present century, experimental work in this field became particularly active, and, with the discovery of vitamin D in 1922, a great impetus was given to studies of the metabolic processes involved.

**103. Interrelation of Calcium, Phosphorus, and Vitamin D.**—Adequate calcium and phosphorus nutrition is dependent upon three factors; a sufficient supply of each element, a suitable ratio between them, and the presence of vitamin D. These factors are interrelated. While an adequate supply of the elements is the first essential, they are more effectively utilized when they are present in a certain ratio to each other. A ration containing 10 parts of calcium to 1 of phosphorus will not provide for efficient assimilation even though the phosphorus is present in what is normally a sufficient amount. The same is true where this relation between the elements is reversed. The normal calcium-phosphorus ratio has been defined as lying between 2:1 and 1:2, but adequate nutrition is possible outside of these limits. The optimum ratio varies somewhat according to the levels of the elements. With plenty of vitamin D in the ration, the ratio becomes of less importance, and more efficient utilization is made of the amounts of the elements present. In the entire absence of the vitamin, assimilation is poor even though the other factors are optimum. The relative importance of these various factors differs considerably in different species. In the present discussion only incidental mention is made of vitamin D since the role of this nutrient is taken up later in Chap. VIII.

**104. The Composition of Bone.**—Approximately 99 per cent of the calcium and 80 per cent of the phosphorus of the body are present in the bones and teeth. Though somewhat variable according to age, state of nutrition, and species, normal adult bone may be considered to have the following approximate composition: water, 50 per cent; ash, 26 per cent; protein, 20 per cent; and fat, 4 per cent. The organic matrix of bone in which the mineral salts are deposited consists of a mixture of proteins, of which the principal one is ossein. The water content of bone decreases with age, and the fat is variable according to the nutritive state since the bone marrow serves as a fat depot, and thus

ash content is expressed most frequently on the basis of the moisture-free, fat-free bone. In mammals the ash is made up approximately as follows: calcium, 36 per cent; phosphorus, 17 per cent; carbon dioxide, 5.5 per cent; and magnesium, 0.8 per cent. There are small amounts of several other minerals.

Assuming that the carbon dioxide is present as calcium carbonate and the magnesium as phosphate, it may be calculated that the ash is made up of approximately 85 per cent of tricalcium phosphate, 14 per cent of calcium carbonate, and 1 per cent of magnesium phosphate. The former belief that bone consists of a simple mixture of these compounds in this relation has been displaced by the view, based on both chemical and X-ray evidence, that the principal constituent is a complex salt of the apatite series. Some state that this salt consists of a combination of calcium carbonate and calcium phosphate, while others believe that it is a hydroxy calcium phosphate and that the calcium carbonate exists separately. Thus, aside from the general agreement that a complex salt is involved, the problem of the chemical nature of bone remains unsolved.

There is little variation in the elementary composition of bone ash. The calcium and phosphorus always occur in approximately a 2:1 ratio, although slight variations with age and diet may occur. Magnesium is somewhat higher than normal in rachitic bone and much lower in magnesium deficiency (Sec. 115). The phosphate-carbonate ratio decreases with age and is lower in rickets. Since bone ash consists almost entirely of calcium and phosphorus salts and since the relative amounts of these elements show little variation, the ash content of bone is commonly used as the measure of its state of calcium and phosphorus nutrition.

Teeth are similar to bone in chemical composition but characteristic differences exist between the enamel, dentine, and pulp. The enamel is the hardest substance in the body and has the lowest water content, approximately 5 per cent. It contains only 3.5 per cent of organic matter.

**105. Calcium and Phosphorus in Soft Tissues.**—The 1 per cent of body calcium which occurs outside the bones is widely distributed throughout the organs and tissues, where it is probably bound, at least in part, to the colloids. The large amounts of phosphorus which are found elsewhere than in the bones are present mostly in organic combinations such as phosphoprotein,

nucleoprotein, phospholipid, phosphocreatine, hexose phosphate, and others. The discussions of these compounds in earlier chapters have indicated their distribution and functions and serve to show the many roles which phosphorus plays in the organism other than as a structural element in bone. Phosphorus makes up 0.15 to 0.2 per cent of the soft tissues of the body.

**106. Calcium and Phosphorus in Blood.**—The blood cells are almost or entirely devoid of calcium, but the serum, in health, contains from 9 to 12 mg. per 100 cc. in most species. In the laying hen, a much higher level occurs during egg production. Two types of serum calcium are distinguished, *diffusible* and *non-diffusible*, differentiated by a membrane impermeable to colloids. The nondiffusible calcium is bound to protein. The diffusible fraction, which makes up 60 per cent or more of the total, is present largely as compounds of phosphate and bicarbonate and is the part which is of principal significance in calcium and phosphorus nutrition. The level of diffusible calcium is not necessarily higher, however, in active metabolism. For example, Taylor and Russell<sup>1</sup> found that while the nondiffusible calcium rose from a level of 6.4 mg. in nonlaying hens to 16.1 mg. during egg production, the diffusible calcium remained constant at 5.4 mg. In addition to its function in bone deposition, the serum calcium is essential for the clotting of the blood and is concerned in the maintenance of acid-base equilibrium. It also plays a role in the physiological balance of basic ions (Sec. 102).

While the primary source of blood calcium is obviously the food, its level is not readily influenced by the dietary intake but is primarily controlled by a hormone from the parathyroid glands. As need arises, this hormone maintains the level in the blood by mobilizing calcium from the bones (Sec. 108). If the parathyroids are removed or fail to function, the blood level drops and *tetany* occurs. This is a hyperirritability of the neuromuscular system which in severe cases results in convulsions. If the glands are abnormally active, as occurs in certain diseases, an excessive mobilization of calcium takes place with a consequent demineralization of the bones. There is an excessive loss of calcium from the body as a result of an increased excretion in the urine. Blood calcium is low in milk fever and usually low in

<sup>1</sup> TAYLOR, M. W., and W. C. RUSSELL, The diffusible calcium in the serum of laying and non-laying hens, *J. Agr. Research*, **51**, 663–667, 1935.

pregnancy disease of sheep. It may be low in rickets, particularly if accompanied by tetany.

Whole blood contains from 35 to 45 mg. of phosphorus per 100 cc., most of which is in the cells. The element occurs in a variety of forms, principally organic combinations. From the standpoint of mineral nutrition, our main interest lies in the inorganic phosphorus which occurs in the plasma, although it is evident that an interchange of phosphate between organic and inorganic forms continually occurs. In health its level generally lies between 4 and 9 mg. per 100 cc., depending upon the age and species. The level is higher at birth than at maturity, the most rapid decline occurring early in life. The maintenance of the inorganic phosphorus level of the blood is governed by the factors which promote calcium and phosphorus assimilation. A low level occurs commonly in rickets. A large number of data on the calcium and inorganic phosphorus content of the blood of various species are presented by Dukes.<sup>1</sup>

The calcium and phosphorus levels in the blood are important indicators of the state of nutrition of these elements; but, in this connection, it is important to remember that their levels represent a balance between several opposing factors: absorption, excretion, deposition, and mobilization. Normal levels do not guarantee a normal state of bone nutrition.

#### **107. Absorption and Excretion of Calcium and Phosphorus.—**

The nutrition of calcium and phosphorus is dependent in the first place upon their absorption and in the second place upon their deposition in the bones. The state of their nutrition is best measured either by balance studies (Sec. 195) which determine the difference between the intake and the outgo in the feces and urine, or by chemical, histological, or X-ray studies of the bones. The absorption of these elements cannot be measured in the same way as is done for organic nutrients because the feces are one of the paths of outgo of the portion which has been absorbed and metabolized, as well as of the portion which escapes absorption. Using special techniques, the process has been studied by taking advantage of the fact that absorption and excretion occur at different places in the intestinal tract. The measurement of the distribution of the minerals between the urine and feces, and the

<sup>1</sup> DUKES, H. H., *The physiology of domestic animals*, pp. 41-45, Comstock Publishing Company, Ithaca, N. Y., 1935.

determination of their concentrations in the blood are also useful in studying absorption.

It is clear that the absorption of both calcium and phosphorus is favored by factors which operate to hold them in solution. An acid medium tends to prevent the formation of the insoluble and thus unabsorbable tricalcium phosphate. Lactose, which promotes an acid reaction in the digestive tract, favors absorption, and assimilation is thereby improved. Certain factors influence the absorption of one element but not the other. Large intakes of iron, aluminum, and magnesium interfere with the absorption of phosphorus by forming insoluble phosphates. An experimentally produced "beryllium rickets" is due to the effect of the beryllium in rendering phosphorus insoluble. Fatty acids may form insoluble calcium soaps which are assimilated with difficulty, yet a certain amount of fat seems to favor the absorption of this element. A great excess of either calcium or phosphorus interferes with the absorption of the other, a fact which helps to explain why a certain ratio between them in the diet is desirable for their best assimilation. With a large excess of either element, the other one tends to become tied up as the insoluble tricalcium phosphate.

Many studies have been made of the influence of crude fiber on the absorption of calcium and phosphorus, with rather conflicting results. In general, the direct addition of cellulose to diets has had little influence, while diets naturally high in fiber appear to have a more marked effect. It is probable that in the latter case the effect is an indirect one, whereby the minerals existing in cells surrounded by fiber which is not digested are thus kept from contact with the absorbing membranes. This is an important practical question, particularly as regards calcium, since the only vegetable foods which are rich in this element are also high in fiber. Where crude fiber or other substances cause a marked laxative effect absorption is reduced.

The measurement of absorption is of limited value because of the fact that absorbed calcium and phosphorus may be promptly excreted unless conditions are favorable for their deposition in the bones. It has been shown, for example, that though the feeding of a mineral acid will increase absorption all of the increase may be rapidly excreted. Thus the nutritionist is more interested in the *net absorption*, the amount which actually remains in the body

as shown by a balance experiment. At best, a large wastage of calcium and phosphorus is involved in their adequate nutrition.

Approximately 75 per cent of the excreted calcium is found in the feces. The figure is somewhat variable according to the diet and other factors, and it is higher in the young than in the adult. Phosphorus is excreted principally in the feces in Herbivora, principally in the urine in Carnivora, and about equally divided between the two channels in man. The distribution between feces and urine can be shown by injecting a salt of the element into the blood stream and noting the increased output in the two

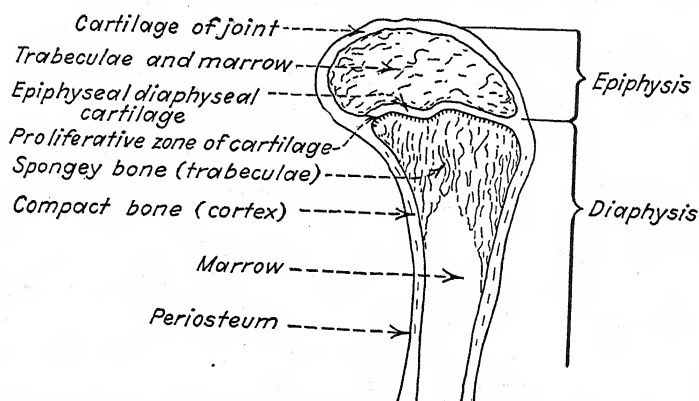


FIG. 8.—Diagram of a longitudinal section of a growing bone. (Courtesy of Katharine Hummel, Cornell University.)

channels. A variety of phosphorus compounds, chiefly phosphates, occur in the urine, of which approximately 6 per cent are in organic form.

#### 108. Deposition and Mobilization of Calcium and Phosphorus.

The bones serve not only as structural elements but also as store-houses of calcium and phosphorus which may be mobilized at times when the assimilation of these minerals is inadequate to meet body needs. Thus the mineral metabolism of bone involves not only the deposition of calcium and phosphorus during growth but also processes of storage and mobilization which occur throughout life. As an aid to an understanding of how these various processes take place, a diagram of a longitudinal section of bone is given in Fig. 8.

The growth of bone in length takes place at the junction of the epiphysis and diaphysis. The cartilage in between is a temporary

formation which grows by the multiplication of its own cells and continues to be replaced at both surfaces by calcified bone. When the cartilage ceases to regenerate and is entirely replaced by bone itself, the epiphysis unites with the diaphysis and growth ceases. This is referred to as the closing of the epiphyses. In the process of ossification, cartilage is converted into ossein and then calcified. The zone where this is taking place is referred to as the proliferation zone of cartilage or the zone of provisional calcification. It is the area which is examined in the "line test" (Sec. 153) used as a measure of the state of calcium and phosphorus nutrition. Bone formation also occurs under the periosteum. At first the only bone formed is of the spongy type. An internal reconstruction is responsible for the formation of the more complex structure called compact bone.

As distinguished from the shaft, the trabeculae are lacelike structures in which a reserve of calcium and phosphorus is deposited for mobilization to meet needs not currently supplied by the diet. This function of the trabeculae has been clearly portrayed by the detailed studies of Bauer, Aub, and Albright.<sup>1</sup> These structures are located close to the epiphyseal ends of the bones where the blood supply is greatest. They provide the calcium mobilized by the parathyroid to maintain the level in the blood. During heavy lactation, they are drawn upon to meet a part of the requirements for the minerals secreted in the milk (Sec. 325). They also may be drawn upon in pregnancy and, perhaps, during periods of rapid growth for the building of the structural portion of the bone. This depletion of the trabeculae involves no physiological harm, and their mineral content is readily restored with an adequate diet during periods when the body needs for calcium are less, for example, during the dry period for the dairy cow. But, if they are not restored, the shaft of the bone may be eventually attacked with serious consequences as is described later.

Like the structure of bone itself, the physicochemical changes involved in the deposition of its calcium salts remain a puzzle, despite much study. It is clear that sufficient concentrations of diffusible calcium and inorganic phosphates are prerequisites. It

<sup>1</sup> BAUER, WALTER, J. C. AUB, and FULLER ALBRIGHT, Studies of calcium and phosphorus metabolism. V. A study of the bone trabeculae as a readily available reserve supply of calcium, *J. Exptl. Med.*, **49**, 145-161, 1929.

is considered that the blood, and thus the fluid bathing the bone, is supersaturated with tricalcium phosphate and that deposition in the bone involves a precipitation of this salt. But ossification is not an inert process of precipitation or else it could occur anywhere in the body. The tricalcium phosphate does not precipitate out in the fluid media but only in active bone and cartilage. It appears that some vital factor of the bony tissue itself must be involved. Robison<sup>1</sup> has produced important evidence that a phosphatase, occurring only in active bone or cartilage, hydrolyzes organic phosphates in the blood cells thus providing an increased concentration of inorganic phosphate ion which causes precipitation to be initiated. In his publication this investigator has reviewed various theories of calcification.

**109. Rickets.**—It is evident from the previous discussion that a failure of normal calcium and phosphorus nutrition may occur at any time of life when the supply of the elements and the factors concerned in their assimilation are not adequate to meet functional needs. In the adult the failure is reflected in a negative balance of the minerals, and, in growth, the balance data show inadequate retention. At both stages there is a decreased ash content of the bones. Their consequent weakening may eventually result in certain external symptoms, such as lameness and fractures, which are alike at all ages, though, during the formative stage, abnormalities of growth which result in misshapen bones are the more common. There are, however, marked differences in the bone pathology, particularly histological, according to the stage of bone development and also according to the specific nutritional deficiency primarily concerned. Therefore, various terms are used to designate different failures of calcium and phosphorus nutrition. Unfortunately there is a lack of uniformity in the use of these terms.

In its broadest sense, rickets represents a disturbance of the mineral metabolism in such a way that the calcification of the growing bone does not take place normally. This is the sense in which the term is used in this book. The cause of the failure lies primarily in the fluid which bathes the bone, rather than in the bone itself, and thus rickets may be considered essentially as a disordered condition of the blood. There is a lowering of the

<sup>1</sup> ROBISON, ROBERT, *The significance of phosphoric acid ester in metabolism*, New York University Press, New York, 1932.



level of inorganic phosphorus or calcium or both in the plasma. The blood picture varies according to the specific dietary deficiency involved. Some authorities limit the use of the term rickets, or *true rickets*, to the specific bone pathology found in very early growth, involving changes which are produced experimentally on a low-phosphorus high-calcium diet deficient in vitamin D and which are accompanied by a low blood phosphorus. There is a widening of the epiphyseal-diaphyseal cartilage, an excessive production of osteoid tissue, and other characteristic



FIG. 9.—An extreme case of rickets caused by a ration low in calcium.

histological changes. But bone abnormalities can develop at any time during growth. They can occur at any time due to a lack of calcium as well as of phosphorus and of the vitamin, and the blood picture may vary as regards the mineral relations. Though the specific bone pathology may differ, the broad definition of rickets includes all of these nutritive failures, recognizing, however, that tetany may also occur if the blood calcium becomes very low. Frequently the terms, *low-phosphorus rickets* and *low-calcium rickets*, are used where a distinction is made.

The failure of bone nutrition during growth results not only in an arrest of its normal development but also in various structural abnormalities. There is an enlargement of the ends of the bones which shows itself in a beading of the ribs and certain other bones. The structural changes cause lameness and stiffness, and, some-

times, actual paralysis due to nerve injury resulting from pressure and even from fractures. In severe and prolonged failure of adequate nutrition, the tension of the muscles pulls the weakened bones out of shape and the weight of the body causes the leg bones to buckle and even to fracture. Such a condition is illustrated in Fig. 9. Rapid growth accelerates the development of rickets. This may be due in part to the demands of muscle formation for phosphorus. The increasing weight of flesh puts an additional strain on the weakening bones and intensifies the external symptoms. As severe rickets develops, however, growth becomes retarded.

Rickets is very common in calves and pigs. Comprehensive reports on the disease in calves are given by Bechdel and coworkers<sup>1</sup> and by Gullickson and associates<sup>2</sup> and the detailed histology is presented by Michigan workers.<sup>3</sup> A low blood calcium appears to be a more marked and regular finding than low phosphorus. In pigs low blood phosphorus is a characteristic finding, but, on low-calcium rations, the blood calcium may be low also. Detailed studies of rickets in this species have been presented by Bethke and coworkers<sup>4</sup> and by Aubel and associates.<sup>5</sup> Rickets has also been reported in sheep, horses, dogs, and several other species. In all of them retardation of growth, inadequate calcification, and bone malformations result.

**110. Osteomalacia.**—The term, osteomalacia, is commonly used to denote a failure of calcium and phosphorus nutrition in the adult bone, and it is so used in this book. A mobilization of calcium and phosphorus salts from the bones of the adult, exceeding the reserve supply present in the trabeculae, results in a breakdown of the structural portion. This excessive mobilization

<sup>1</sup> BECHDEL, S. I., K. G. LANDSBURG, and O. J. HILL, Rickets in calves, *Pa. Agr. Expt. Sta. Tech. Bull.* 291, 1933.

<sup>2</sup> GULLICKSON, T. W., L. S. PALMER, and W. L. BOYD, A rickets-like disease in young cattle, *Minn. Agr. Expt. Sta. Tech. Bull.* 105, 1935.

<sup>3</sup> BECHTEL, H. E., E. F. HALLMAN, C. F. HUFFMAN and C. W. DUNCAN, Pathology of rickets in dairy calves, *Mich. Agr. Expt. Sta. Tech. Bull.* 150, 1936.

<sup>4</sup> BETHKE, R. M., B. H. EDGINGTON, and C. H. KICK, Effect of the calcium-phosphorus relationship of the ration on growth and bone formation in the pig, *J. Agr. Research*, 47, 331-338, 1933.

<sup>5</sup> AUBEL, C. E., J. S. HUGHES, and H. F. LIENHARDT, The effects of low phosphorus rations on growing pigs, *J. Agr. Research*, 52, 149-159, 1936.

may be caused by an overfunctioning of the parathyroid or by some other pathological condition, but it is due most frequently to a continued body demand for calcium and phosphorus which is greatly in excess of the supply being assimilated. Most of the acute cases occur during pregnancy and lactation when, owing to an inadequate diet, excessive demands are made upon bones already depleted. Any adult animal which continually fails to receive calcium and phosphorus nutrition adequate for its needs must gradually deplete its bones. Whether this occurs to the point where the bones break down or production becomes lessened depends upon the extent of the deficiency.

A striking depletion of the bones which resulted in broken pelvis in dairy cows continuously fed on calcium-deficient roughages has been described by Becker, Neal, and Shealy.<sup>1</sup> A similar condition caused by phosphorus deficiency has been reported from South Africa and elsewhere. Osteomalacia in horses on a low-calcium high-phosphorus ration has been described by Philippine workers.<sup>2</sup> The trouble has been reported in sheep, beef cattle, swine and other species. A characteristic feature of the trouble is a negative calcium and phosphorus balance. The blood may be low in one or both of the minerals. If very low in calcium, there may be tetany also. Provided it is not too far advanced, osteomalacia responds to treatment by correcting the dietary deficiency which is responsible for the inadequate calcium and phosphorus nutrition of the bones. Clearly the necessity of giving attention to bone nutrition does not end with the close of the growth period. An active metabolism continues throughout life, and, for a normal productive life of breeding stock and lactating animals, this nutrition must be such as to insure that the bones do not become depleted, even though acute symptoms of osteomalacia do not occur.

Like rickets, osteomalacia is a term used in somewhat different senses by different authorities. In reading accounts of investigations dealing with either of these troubles, it is important to note how the terms are being used. *Osteoporosis* is another term that

<sup>1</sup> BECKER, R. B., W. M. NEAL, and A. L. SHEALY, Effect of calcium-deficient roughages upon milk production and welfare of dairy cows, *Fla. Agr. Expt. Sta. Tech. Bull.* 262, 1933.

<sup>2</sup> KINTER, J. H., and R. L. HOLT, Equine osteomalacia, *Philippine J. Sci.*, 49, 1-89, 1932. SUMULONG, MANUEL D., Observations on the bones of native horses affected with osteomalacia; *ibid.*, 53, 141-158, 1934.

has a rather variable meaning. Those who consider the low-phosphorus variety as the only true rickets employ it to denote the histology where low calcium is primarily involved. *Aphosphoresis* and *acalciosis* denote, respectively, a condition due to a lack of phosphorus and a condition due to a lack of calcium. The role of vitamin D in these variously named troubles is discussed later (Sec. 149).

Much of our knowledge of the effects of phosphorus deficiency is due to the work of Theiler and associates<sup>1</sup> in South Africa.

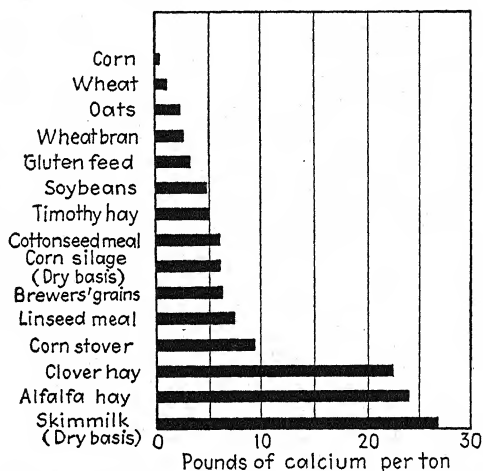


FIG. 10.—Calcium content of common feeding stuffs.

They showed that large losses which occurred in both growing and adult cattle on the range were due to a very low content of the element in the herbage as a result of a deficiency in the soil, a situation which has since been noted in other parts of the world including various areas in the United States. Though less widespread, similar areas of calcium deficiency have been reported to exist in certain parts of the world.

**111. The Calcium and Phosphorus Content of Feeds.**—The different concentrates and roughages vary widely in their content of calcium and phosphorus. Certain combinations furnish a sufficient supply of these minerals while others are deficient. Thus an important aid in providing for adequate calcium and

<sup>1</sup> THEILER, ARNOLD, H. H. GREEN, and P. J. DU TOIT, Phosphorus in the live stock industry, *J. Dept. Agr., Union. S. Africa*, 8, 460-504, 1924.

phosphorus nutrition is a general knowledge of the composition of the common feeds. This knowledge enables the feeder to consider minerals, as well as protein and total digestible nutrients, in making up his rations and provides him with the information for determining when supplementary sources of these elements are needed.

The relative amounts of calcium in certain typical feeds are shown in Fig. 10. For comparative purposes the values for skim milk and corn silage are given on a dry basis. The cereal

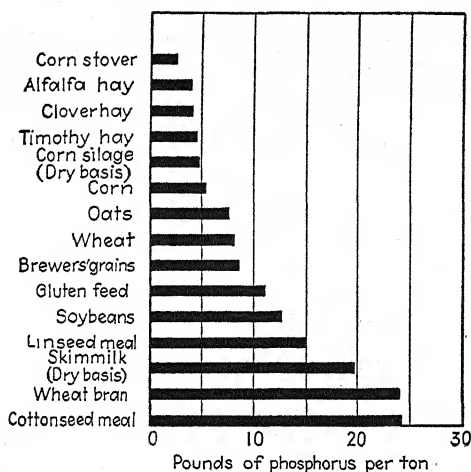


Fig. 11.—Phosphorus content of common feeding stuffs.

seeds are all low in calcium. Legume seeds, notably soybeans, are higher, and the same is true for the oil meals. All seeds and their products, however, must be classed as poor sources of calcium in terms of the requirement of the animal body. Grass hays, such as timothy, are also poor in contrast to legume hays which are rich. On a dry basis, skim milk exceeds the best hays in calcium content. Much richer than any of the feeds shown in the chart, however, are the animal by-products containing bone, such as tankage, meat scrap, and fish meal. A 60 per cent protein tankage will furnish four or five times as much calcium as will legume hay or skim milk and twenty times as much as will vegetable protein concentrates such as the oil meals.

As is shown in Fig. 11, these same feeds present a very different picture in phosphorus content. Here the seeds are uniformly

higher than the roughages, and seed by-products, such as wheat bran and the oil meals, are especially rich in phosphorus. Skim milk is the only feed included in the charts which can be classed as rich in both calcium and phosphorus. The bone-carrying animal by-products are also very rich in both elements. Tankage supplies more than twice as much phosphorus as any feed shown in Fig. 11. The milled flours are lower in both calcium and phosphorus than the whole seeds.

While the data presented in Figs. 10 and 11 are useful to show the differences between the various kinds of feeds, they must not be considered to be exact values, because the calcium and phosphorus contents of feeds, especially the roughages, are variable according to the nature of the soil on which they are grown, the fertilizer used, and the water relations. Timothy hay grown on fertile soil may contain two or three times as much calcium as that grown on a worn-out acid soil. On the other hand, legume hays require a soil rich in lime for satisfactory growth, and thus, while they show some variation according to soil, they can always be relied upon as rich sources of calcium. All hays are highly variable in phosphorus content according to the supply available in the soil and to other factors. In a recent study of fifty samples of timothy hay grown on different soils in central New York, the calcium ranged from 0.14 to 0.34 per cent and the phosphorus, from 0.13 to 0.29 per cent. Thus, the feeder must know something about the mineral content of his roughage in order to tell the exact conditions under which he needs a mineral supplement. General statements that one type of hay always needs a supplement, while another type never does, cannot always hold. Pasture grass is subject to even larger variations than dry roughage because the species of the grasses is a large factor and because climatic conditions also play a role.

**112. Availability of Calcium and Phosphorus of Feeds.**—Little is known about any differences in availability of the calcium and phosphorus of the various feeds. Half or more of the phosphorus of mature cereal seeds and their products, notably wheat bran which is an especially rich source of the element, is present as phytin, a cyclic compound in which inositol,  $C_6H_6OH_6$ , and phosphoric acid are combined. Leaves and stems apparently have little or none. Several experiments have shown that this phytin phosphorus is poorly utilized by rats and children, a fact which

apparently explains the observations that certain cereals, notably oatmeal, are poor sources of phosphorus for healing rickets experimentally produced on a high-calcium low-phosphorus diet. This observation is an example of what has been referred to as the "anticalcifying action" of cereals. McCance and Widdowson<sup>1</sup> have reviewed the literature of this subject and presented data showing that in man 20 to 50 per cent of the phytin of cereals is excreted unchanged in the feces. Since farm animals normally receive most of their phosphorus from cereals, the question of their ability to utilize phytin is an important one. While there is evidence suggesting that cattle and swine can utilize it fairly well, further studies are needed.

Several studies with children have shown that the calcium of vegetable materials is not so well utilized as the calcium of milk. The influence of fiber in lowering digestibility and absorption may be concerned here. This influence should be of less significance in the case of farm animals, especially the Herbivora. It is clear that the latter can make effective use of the calcium of leafy materials.

A recent finding of special interest is that the calcium of leafy materials which contain appreciable amounts of oxalic acid is poorly utilized. Several studies have shown this to be true for spinach, one of the richest plant sources of calcium. Fincke and Sherman<sup>2</sup> have proved that this poor utilization is due primarily to its high oxalic acid content. The spinach calcium was little utilized if at all, while the calcium of kale, which contains practically no oxalic acid, was nearly as well assimilated as that in milk. Oxalic acid may interfere with calcium absorption by forming the highly insoluble calcium oxalate and it also may exert a deleterious effect in the blood stream. Several experiments have shown that the injection of soluble oxalates reduces blood calcium, causing tetany which may terminate in death.

In so far as analytical data are available, it would appear that the high oxalic acid content of spinach is not shared by leafy vegetables generally. Regarding hays and other forage crops there is a lack of information. The desirability of giving this matter

<sup>1</sup> McCANCE, R. A., and E. M. WIDDOWSON, Phytin in human nutrition, *Biochem. J.*, **29**, 2694-2699, 1935.

<sup>2</sup> FINCKE, M. L., and H. C. SHERMAN, The availability of calcium from some typical foods, *J. Biol. Chem.*, **110**, 421-428, 1935.



attention is indicated by reports that decalcification has been noted in sheep grazed on feed containing oxalic acid. These recent findings regarding oxalic acid serve to emphasize the fact that the physiological significance of the organic acids of foods is an important field which has been little studied. It has a special importance in the case of herbivorous animals.

**113. Phosphorus Deficiency and Appetite.**—A deficiency of phosphorus has a specific effect in causing a loss of appetite, and

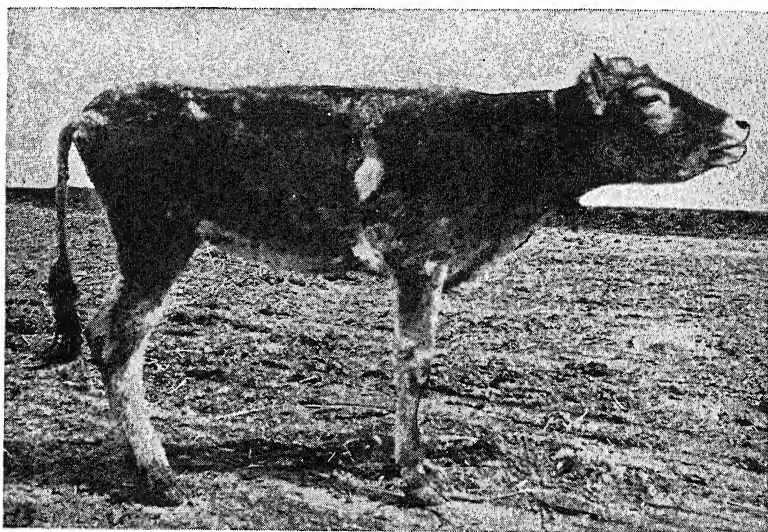


FIG. 12.—“Wood-chewing” calf which developed this depraved appetite while on a ration of grain and poor hay, a ration low in phosphorus and vitamin D.

even a depraved appetite, frequently referred to as “pica,” which is exhibited in the eating of bones, wood, clothing, and other materials to which the animal may have access. The animal becomes very emaciated. This condition, which is illustrated in Fig. 12, is most frequently met in grazing animals in areas where the soil, and thus the forage, is very low in phosphorus. It may also occur in barn feeding and here a lack of vitamin D may also be involved. Very large losses have occurred among grazing animals in different parts of the world as a result of this severe phosphorus deficiency, even where the forage was abundant and nutritionally adequate in other respects. Many of the deaths have resulted from diseases to which the weakened animals become especially susceptible, notably from



infections contracted by eating decaying bones of animals which had died. Bone meal or other rich sources of phosphorus are now being effectively used to prevent these losses.

The fact that phosphorus deficiency results in a severe emaciation has raised the question as to whether there is a poorer utilization of the small amount of food consumed. In studying this question with dairy cattle, Riddell, Hughes, and Fitch<sup>1</sup> found no decrease in digestibility, but there was evidence of a higher energy metabolism. From a critical experiment with beef heifers, Kleiber, Goss, and Guilbert<sup>2</sup> concluded that loss of appetite was the main factor but that there was a slightly lower efficiency of energy utilization.

**114. Calcium and Phosphorus Supplements.**—In selecting rations for their calcium and phosphorus content, consideration should first be given to supplying the minerals, in so far as possible, by choosing those feeding stuffs that are rich in the elements needed. However, the farmer must build his rations around the available feeding stuffs on his own farm, and it may not be practicable for him to make up his rations so that they will be rich in the needed minerals. Wherever it is not practicable or possible to provide for adequate mineral nutrition by an appropriate combination of the feeding stuffs available, mineral supplements should be used.

The qualities which determine the feeding value of a supplement are: content of calcium and phosphorus, fineness of division, and freedom from harmful impurities. The analyses of commonly available calcium and phosphorus supplements are given in Table XII. The chemical composition of these supplements varies according to the purity of the raw material and the method of processing. Standard products should have a composition approximating that represented by the figures given in the table. The figure for ground limestone is representative of the high-calcium products available. Dolomitic limestones have much smaller and variable contents of calcium.

<sup>1</sup> RIDDELL, W. H., J. S. HUGHES, and J. B. FITCH, The relation of phosphorus deficiency to the utilization of feed in dairy cattle, *Kan. Agr. Expt. Sta. Tech. Bull.* 36, 1934.

<sup>2</sup> KLEIBER, MAX, HAROLD GOSS, and H. R. GUILBERT, Phosphorus deficiency metabolism and food utilization in beef heifers, *J. Nutrition*, **12**, 121-153, 1936.

Many studies have shown that the minerals of these supplements are readily utilizable by both animal and man. Assuming that no harmful substances are present in the supplements and that the animals are in a suitable physical condition, all may be considered to be of equal value per unit of calcium and phosphorus present. These elements in a rock product are utilized by the animal substantially to the same extent as are the same elements

TABLE XII.—PERCENTAGE COMPOSITION OF CALCIUM AND PHOSPHORUS SUPPLEMENTS

Supplements	Calcium	Phosphorus
Raw bone meal.....	22.5	10.5
Steamed bone meal.....	31.3	14.4
Dicalcium phosphate.....	23.5	18.7
Spent bone black.....	22.0	10.9
Ground limestone.....	38.5	
Oyster shells.....	37.9	

in bone or in soluble or organic salts. While the minerals must be in solution in order to be absorbed, the factors which determine solubility in the intestine are more important than the form ingested. It is evident, however, from the recent work of Deobald and associates<sup>1</sup> with chicks, that the calcium of highly insoluble, complex iron-calcium silicates is not utilized. While much of the phosphorus in the body is in organic compounds, inorganic phosphorus can be utilized in their synthesis.

#### MAGNESIUM

Though present in the body in a much smaller amount, the magnesium is closely associated with calcium and phosphorus, both in its distribution and in its metabolism. Approximately 70 per cent of the body supply is in the skeleton, the remainder being found widely distributed in the various fluids. Blood serum contains 1 to 3 mg. of the element per 100 cc. and its level tends to vary with the phosphorus content. The considerable percentage which is found elsewhere than in the bones indicates that its distribution in the body as a whole follows that of phosphorus rather than of calcium. In fact, though the calcium con-

<sup>1</sup> DEOBALD, H. J., C. A. ELVEHJEM, E. B. HART, and J. G. HALPIN, Availability of calcium salts for bone formation and rickets prevention in chicks, *Poultry Sci.*, 15, 42-47, 1936.

tent of the entire body is many times that of magnesium, the soft tissues actually contain much more of the latter. Like calcium and phosphorus, magnesium is excreted in both the urine and feces. The major output is found in the feces.

**115. Symptoms of Magnesium Deficiency.**—Our knowledge that magnesium is an essential element is due primarily to the work of Kruse, Orent, and McCollum.<sup>1</sup> Lowering its content in the diet to 1.8 parts per million resulted, in rats, in vasodilation, hyperirritability, convulsions, and death. In the main the same symptoms occurred in dogs. A characteristic blood finding was a lowered magnesium content but normal calcium and phosphorus. This picture led the investigators to call the trouble "magnesium tetany," thus distinguishing it from the usual tetany in which a low blood calcium is characteristic. In magnesium deficiency, the bones are lower in this element, but they have a higher than normal content of ash, especially of calcium.

The work of Kruse and associates has focused attention on a disease of cattle called "grass tetany" or "grass staggers." This disease has been reported especially from Holland and also from New Zealand as a sickness which occurs in fresh cows within a week or two after they are turned to pasture. It has been comprehensively studied by Sjollem.<sup>2</sup> The physical symptoms are similar to those of magnesium tetany, there is a similar frequency of fatal outcome and the blood magnesium is low. The picture is complicated, however, by the fact that the blood calcium is generally low also. Though it seems probable that a failure of magnesium nutrition is concerned in grass tetany, further studies are needed before this view can be held with any certainty.

A tetany in calves, characterized by a low blood magnesium with normal calcium and phosphorus, has been recently reported by Duncan, Huffman, and Robinson<sup>3</sup> of the Michigan Experiment

<sup>1</sup> KRUSE, H. D., E. R. ORENT, and E. V. MCCOLLUM, Studies on magnesium deficiency in animals. I. Symptomatology resulting from magnesium deprivation, *J. Biol. Chem.*, **96**, 519-539, 1932; III. Chemical changes in the blood following magnesium deprivation, *ibid.*, **100**, 603-643, 1933.

<sup>2</sup> SJOLLEMA, B., On the nature and therapy of grass staggers, *Vet. Rec.*, n.s., **10**, 425-430, 450-453, 1930.

<sup>3</sup> DUNCAN, C. W., C. F. HUFFMAN, and C. S. ROBINSON, Magnesium studies in calves. I. Tetany produced by a ration of milk or milk with various supplements, *J. Biol. Chem.*, **108**, 35-44, 1935.

Station. The calves were reared for extended periods, either on milk alone, a food which is rather low in magnesium, or on milk plus special supplements which contained little or none of the element. In a continuation of these studies by the Michigan workers, the effectiveness of magnesium oxide in maintaining a normal blood level of this element has been shown, and the gross and microscopic pathology associated with low blood magnesium in calves has been described. They report that 10 mg. of magnesium per kilogram of body weight is an adequate allowance. Daniels and Everson<sup>1</sup> give the magnesium requirement for children as 13 mg. per kilogram.

Magnesium is recognized to be concerned in several specific body functions. It activates the enzyme, phosphatase, and it plays a role in carbohydrate metabolism. The element is essential for the proper formation of both the teeth and their supporting structures. It has been ascribed a helpful role in rickets by some investigators and a harmful role by others. There is a possibility that it exerts a variable effect here, depending upon the level fed and the accompanying calcium and phosphorus relations.

So far as is known there is no magnesium deficiency in the usual rations of farm animals, though the experiments with cattle and calves previously cited indicate the desirability of specific studies with other species. Most of the commonly fed roughages and concentrates contain at least 0.1 per cent of magnesium and many contain three or four times this figure. So far as the requirements have been studied, it would appear that a ration containing 0.1 per cent should provide considerably more than is needed.

**116. Calcium and Magnesium Interrelationships.**—In the nutrition of farm animals, much attention has been given to the question of the possible deleterious effects of excessive magnesium intakes in view of its high level in certain waters and in relation to the use of dolomitic limestone as a calcium supplement. Early observations that the injection of magnesium salts exerted certain inhibitory effects on body functions were extended by Loeb and especially by Meltzer and Auer<sup>2</sup> in showing that calcium antago-

<sup>1</sup> DANIELS, A. L., and G. J. EVERSON, A study of the magnesium needs of preschool children, *J. Nutrition*, **11**, 327-341, 1936.

<sup>2</sup> MELTZER, S. J., and J. AUER, The antagonistic action of calcium upon the inhibitory effect of magnesium, *Am. J. Physiol.*, **21**, 400-419, 1908.

nized these inhibitory effects. A year later Mendel and Benedict<sup>1</sup> reported that injecting calcium increased the urinary output of magnesium and, more important, that the injection of magnesium caused large urinary losses of calcium as an accompaniment of the pathological symptoms. Later workers found that magnesium interfered with calcification in vitro, an effect which was overcome by adding phosphorus. These various observations naturally raised the question as to whether a high dietary intake of magnesium had a deleterious effect on calcium assimilation, and some positive evidence for this view was suggested by early work.

It has become apparent, however, from several carefully controlled studies with different species that, when the magnesium is ingested instead of injected, there is a much smaller loss of calcium or no loss at all, due to a selective absorption which prevents most of the magnesium from entering the blood stream. It also appears that the extent of the absorption and thus of the deleterious effect on calcium retention is governed by the amount of calcium and phosphorus, particularly the latter, in the ration. When these two elements are present in liberal amounts the harmful effect of the magnesium is slight or nil. Palmer, Eckles, and Schutte<sup>2</sup> for example, have shown that the ingestion of magnesium sulfate by cattle on a low-phosphorus diet results in serious and continuous losses of calcium which are overcome by increasing the phosphorus content of the ration.

Recent experiments with pigs, chickens, and rats, using rations liberal in phosphorus, have shown that dolomitic limestone is a satisfactory source of calcium for bone formation despite its magnesium content. Thus it appears that, provided both calcium and phosphorus are plentifully supplied, the ingestion of at least a moderate excess of magnesium, either in a mineral supplement or in water or other foods, will not markedly disturb calcium retention, though it may tend to increase the requirements for calcium and phosphorus in the ration. Further studies of this question may be worth while, and certainly it should be remembered that a magnesium limestone is of value as

<sup>1</sup> MENDEL, LAFAYETTE B., and S. R. BENEDICT, The paths of excretion for inorganic compounds. IV. The excretion of magnesium; V. The excretion of calcium, *Am. J. Physiol.*, **25**, 1-22, 23-33, 1909.

<sup>2</sup> PALMER, L. S., C. H. ECKLES, and D. J. SCHUTTE, Magnesium sulfate as a factor in the retention of calcium and phosphorus in cattle, *Proc. Soc. Exptl. Biol. Med.*, **26**, 58-62, 1928.

a calcium supplement only in proportion to its content of this element and that magnesium is at best simply inert material.

Further evidence of the interrelationship between calcium and magnesium is presented by the recent finding of Tufts and Greenberg<sup>1</sup> who showed that a larger amount of magnesium is required to prevent the symptoms of deficiency of this element as the calcium intake is increased. Javillier<sup>2</sup> has reviewed the literature on the significance of magnesium in animal and plant nutrition and published tables of analyses of plant and animal material.

### SODIUM, POTASSIUM, AND CHLORINE

In contrast to the minerals previously discussed which are found principally in the bones, sodium, potassium, and chlorine occur almost entirely in the fluids and soft tissues of the body, where they serve to maintain osmotic pressure and acid-base equilibrium and where they play important roles in water metabolism.

**117. Sodium.**—The body contains approximately 0.2 per cent of sodium. It occurs primarily in the extracellular fluids, in contrast to potassium, which is found largely within the cells. Sodium makes up 93 per cent of the bases of blood serum, and thus it is the predominant basic element concerned in neutrality regulation. The element seems to be absent from blood cells, but it does occur in considerable amounts in the muscles, where it is associated in some unknown way with their contraction. The need for sodium becomes readily evident in the retarded growth that results on diets low in the element. A lack of sodium also lowers the utilization of digested protein and energy and prevents reproduction. In laying hens, a deficiency results in lowered production, loss of weight, and cannibalism.

Sodium salts are readily absorbed and circulate throughout the entire body. Since the element is not stored appreciably, any excess intake is rapidly excreted. Normally over 90 per cent of the excretion takes place through the kidneys as chlorides and phosphates. There is some loss in the perspiration, which at

<sup>1</sup> TUFTS, ELMA V., and DAVID M. GREENBERG, Calcium involvement in magnesium deficiency, *Proc. Soc. Exptl. Biol. Med.*, **34**, 292-294, 1936.

<sup>2</sup> JAVILLIER, M., Le magnésium et la vie; le magnésium engrais et le magnésium aliment, *Bull. soc. chim. biol.*, **12**, 709-740, 1930.

hard work, particularly in warm weather, may represent by far the major portion of the total excretion.

**118. Potassium.**—The body contains somewhat more potassium than sodium. That potassium is primarily a cellular constituent is illustrated by the fact that human blood cells contain over twenty times as much of the element as does the plasma. It plays a vital but little understood role in muscle where its content is six times that of sodium. While blood plasma contains many times as much sodium as potassium, in milk the reverse is true. The reason for the very different distribution throughout the body of these two elements which are so closely related chemically is a mystery.

Though it can be readily demonstrated by growth experiments that potassium is an essential mineral, all foods, particularly plant products, are so rich in it that there is never any deficiency in the diet. The element, like sodium, is readily absorbed and the excess over body needs is immediately excreted. This excretion normally takes place in the urine to the extent of 90 per cent, but profuse sweating diverts a large portion through this channel.

*Sodium-potassium Ratio.*—The fact that plant products contain many times as much potassium as of sodium early raised the question as to the significance of the sodium-potassium ratio in the diet. In 1873 Bunge,<sup>1</sup> on the basis of rather meager data, evolved the theory that an excessive intake of potassium impoverished the organism of sodium and chlorine. This theory was extended to explain the apparently larger requirement of Herbivora than of other species for common salt, as being due to the great excess of potassium relative to sodium in leafy materials. Pasture grass, for example, may contain eighteen times as much potassium as sodium. This attractive theory, which has continued to receive attention, has been supported by the results of some of the later studies, while, in others, the findings have been entirely negative. The more reliable evidence indicates that,

<sup>1</sup> Gustave von Bunge (1844–1920), trained both in chemistry and in medicine, had a long and outstanding career as a teacher and investigator, serving for many years as professor of physiological chemistry at Basel. He made many contributions to the knowledge of the nutrition of minerals notably iron, and wrote a textbook, "Physiologie des Menschen," which contains a wealth of information for the modern student.

though a high-potassium intake may result in an initial increased excretion of sodium from the body, some adjustment rapidly occurs whereby this increased loss is stopped. Experiments with cows given free access to salt have not shown any relation between the amount of salt eaten and the variations in the potassium intake.

**119. Chlorine.**—Differing from sodium and potassium, chlorine is found in large concentrations both within and without the cells of the body tissues. Blood cells contain about one-half as much as the plasma. Approximately 15 to 20 per cent of the chlorine of the body appears to be in organic combination. The chlorides of the blood, principally sodium chloride, make up two-thirds of its acidic ions, which indicates its large role in acid-base relations. The gastric secretion contains chlorine as free acid and in the form of salts. The body has a certain capacity to store chlorine in the skin and subcutaneous tissues. Its excretion follows that of sodium and potassium.

**120. The Use of Common Salt.**—The age-old practice of including common salt in the diets of both man and animals means that most of the sodium and chlorine is both ingested and excreted in this form. Since salt serves as a condiment as well as a nutrient, the intake tends to be highly variable and frequently in excess of needs. Its use as a condiment has physiological support in evidence that it stimulates salivary secretion and promotes the action of diastatic enzymes. When the intake is at a minimum, the body makes an adjustment whereby the output of sodium and chlorine in the urine nearly ceases. The same is true for potassium. In contrast, large intakes involve a correspondingly large excretion, the water requirement being increased accordingly. The kidney is the regulating organ which, through its secretory activity, controls the concentration of electrolytes in the blood.

In 1905 Babcock<sup>1</sup> reported a long-time study of the role of salt in the dairy ration. He found that cows receiving no salt exhibited an abnormal appetite for it after two or three weeks but that a much longer time elapsed, even a year, before any ill effect on health was noted. Eventually there was a loss of appetite, an unthrifty condition, and a marked decline in weight and milk

<sup>1</sup> BABCOCK, S. M., The addition of salt to the ration of dairy cows, *Wis. Agr. Expt. Sta. Ann. Rept.* 22, 129-156, 1905.



yield. These symptoms appeared first in the higher producers, and the breakdown most frequently occurred at calving or shortly after at the height of milk flow. The feeding of salt produced rapid recoveries in animals showing acute symptoms. The long period which elapsed before health was affected by salt deprivation illustrates the ability of the body to husband its supply of sodium and chlorine, reducing their excretion to a minimum, when the intakes are very small. Babcock expressed the view that dry cows and steers would suffer no great inconvenience if given no salt except that contained in a normal ration.

The human kidney may excrete as little as 1 g. or as much as 40 g. of sodium chloride per day depending on the intake. Given normal kidneys and an appropriate water intake, large amounts can be excreted without harm. Excessive intakes, however, result in water retention in the body causing oedema. Among farm animals, the chicken has been considered to have a low tolerance for salt, yet Mitchell and coworkers<sup>1</sup> found that they could be reared from 9 to 21 weeks of age on rations containing 8 per cent of salt. Farm animals have died, however, from consuming salt when given free access to it following a long period of salt deprivation or partial starvation. The tolerance of the animal body for potassium is apparently somewhat less than for sodium.

The salt requirement is greatly increased under conditions which cause heavy sweating because of the large loss in this secretion. Miners have been noted to lose  $2\frac{1}{2}$  kg. of sweat per hour, containing 2 g. of sodium chloride. If large amounts of water are drunk under these conditions cramps result. The cramps disappear on drinking water containing salt.

An excellent detailed statement of sodium, potassium, and chloride metabolism is given by Peters and Van Slyke.<sup>2</sup>

#### IRON AND COPPER

Although the body contains only about 0.004 per cent of iron, this element plays a central role in life processes. As a con-

<sup>1</sup> MITCHELL, H. H., L. E. CARD, and G. G. CARMAN, The toxicity of salt for chickens, *Ill. Agr. Expt. Sta. Bull.* 279, 1926.

<sup>2</sup> PETERS, J. P., and D. D. VAN SLYKE, Quantitative clinical chemistry, Vol. I, Chaps. 15, 19, Williams & Wilkins Company, Baltimore, 1931.

stituent of the respiratory pigment, hemoglobin, iron is essential for the functioning of every organ and tissue of the body. It plays a fundamental role as a catalyst for cellular oxidation. Most of the iron present in the body is in the form of hemoglobin. In addition to small amounts in other active forms distributed throughout the body, a variable store is located in the liver and, secondarily, in the spleen and kidneys. Since the red cells and their hemoglobin are constantly being destroyed and replaced, iron obviously undergoes a very active metabolism. Its synthesis into hemoglobin occurs throughout life as well as during the growing period when the total blood supply is being augmented. For hemoglobin synthesis, a small amount of copper is necessary, though this element is not a constituent of the hemoglobin itself. This role of copper, discovered by Hart and associates of the University of Wisconsin, establishes it as an essential mineral element. The copper present in the body is only a small fraction of its iron content, but it is apparently rather widely distributed in the tissues, notably in the liver. It is present in blood though not in the hemoglobin.

**121. Hemoglobin Formation.**—The blood cells which contain the hemoglobin are formed in the bone marrow. It is considered that these red corpuscles are destroyed after an average life of four to six weeks. In the course of their destruction, the heme of the hemoglobin is split into an iron compound and bilirubin and other pigments which are carried to the liver and secreted in the bile. The principal channel of iron excretion is the feces. Iron released by the normal blood cell destruction can be used again to form hemoglobin, but, in certain diseases, this destruction may be accelerated and iron formed by toxic destruction cannot be reutilized. If the cells are not renewed so rapidly as destroyed, or, if the increase in the number of cells which are required to enlarge the blood supply with growth does not occur, *anemia* results. The condition of the blood in this respect is commonly determined by measuring its hemoglobin content. The normal content of hemoglobin for most mammals lies within the range, 10 to 15 g. per 100 cc. of blood. In severe anemia the value may drop to one-half or even a third of the normal.

The physiologist distinguishes several different types of anemia. The importance of iron as a factor varies with the type. In *pernicious anemia*, also called *primary anemia*, there is no lack of

iron or failure of hemoglobin formation but rather a lack of a substance essential to stimulate the production of the red corpuscles. Perhaps this substance acts specifically on the bone-marrow cells. Additional iron or iron and copper are of no benefit. The active substance is present in liver and thus pernicious anemia sufferers can be kept alive by the consumption of liver or of liver extract, but no real cure results for the substance must be supplied constantly. An extract of stomach tissue is also effective and a combination of liver and stomach extracts is more effective than liver alone. In pernicious anemia there is a lack of hydrochloric acid in the gastric juice and a gastric disturbance is considered a primary cause of the trouble. For their discoveries leading to this knowledge of the cause and treatment of pernicious anemia, which previously proved fatal to man in about three years, Whipple, Minot, and Murphy received the Nobel prize in medicine. This type of anemia is not known to occur in farm animals.

*Secondary anemias* are due to a loss of blood as in hemorrhage, or to accelerated blood destruction in various diseases. Both mineral and organic factors are concerned in blood regeneration in these anemias. They can be cured only by eliminating the cause.

**122. Nutritional Anemia.**—While nutrition is concerned in the treatment of the previously described anemias, the term, *nutritional anemia* refers to those caused primarily by a lack of iron and the associated essential mineral, copper. Nutritional anemia may occur at any time of life when the available supply of the minerals becomes deficient relative to the needs for hemoglobin formation. It is particularly likely to develop in certain species during the suckling period, since milk is very low in iron. Our knowledge of the iron nutrition during this period is due particularly to the early work of Bunge and Abderhalden. A comparison of the mineral constituents of milk and of the newborn showed that, while the other constituents were in similar concentrations, the percentage of iron in the milk ash was only one-sixth of the figure for the iron in the ash of the newborn. It was also found that there was a much larger percentage of iron present at birth than later in life. The explanation was therefore made that nature provides for the iron requirements of the suckling largely by means of a store in its body at birth which may be drawn upon

for blood formation and other essential functions during the period when milk is normally the principal or sole food. Bunge found that the guinea pig, which normally commences to eat leafy material within a day after birth, is born without any special store of iron.

This provision of nature for iron nutrition in the suckling does not always prove adequate. The store in the newborn is influenced by the diet of the mother during gestation. If the birth occurs prematurely there is a smaller store because most of the storage occurs late in gestation. If the number of young born is larger than normal for the species, for example, twins in humans and extra large litters in hogs, the individual's supply tends to be smaller. Even if the store is normal, a long nursing period without supplementary iron-rich food may exhaust it. The reserve of the human young is usually exhausted before the end of the sixth month. These factors are responsible for many cases of nutritional anemia in babies. In farm animals, the trouble occurs as a practical problem only in the case of pigs. In both species the anemia can be prevented by the feeding of iron and a trace of copper to the sucklings. Without copper the iron is absorbed and stored in the liver but hemoglobin synthesis does not occur. It does no good to feed the minerals to the lactating mother, for the iron content of milk cannot be increased in this way. A detailed description of this anemia in suckling pigs and its treatment is given later (Sec. 273).

While anemia has been produced experimentally in lambs and calves by restricting them to a milk diet, in practice the disease is not met in them because they early begin to eat supplementary food which supplies the needed iron. Nutritional anemia can occur beyond the suckling stage, at any time of life when the supply of iron in the food is inadequate to meet the needs for hemoglobin formation. In the case of farm animals, however, the commonly fed rations are so rich in iron that the trouble does not occur except in very special situations. Such a situation exists in Florida where the soil is very poor in iron. Cattle receiving a ration consisting mostly or entirely of grass forages grown on this soil become anemic but respond to the iron and copper treatment. Anemia has been reported in range animals in certain other parts of the world, but further proof is needed that a lack of iron is the sole cause.

**123. The Iron Value of Foods.**—Aside from milk, most of the feeds for animals contain very liberal amounts of iron relative to the need by the body. Leafy materials are rich sources and so are many seeds. Since most of the iron in cereal seeds is in the outer coatings and germ, milling results in increasing the supply to animals, but in decreasing it in so far as man is concerned. It is recognized, particularly because of the recent work of Elvehjem and associates,<sup>1</sup> that the availability of iron differs according to its food source. According to these workers, hematin iron is totally unavailable, while certain inorganic salts are completely utilized. Among the natural foods, liver, heart, and soybeans and certain other legume seeds contain highly available iron, ranking somewhat above muscle tissue, oats, and wheat. On the other hand, the large amount of the element which is present in such leafy materials as alfalfa and spinach is poorly utilized. The method used by Elvehjem and associates in obtaining these results is a chemical one. While some uncertainty remains as to how closely the results represent biological performance in all cases, the investigations show clearly that wide differences in availability exist.

In so far as farm animals, including chickens, are concerned their usual rations are so rich in iron that they can normally be counted upon to supply enough even though its availability is low. Aside from the special situations previously referred to, the addition of iron salts to the rations of various species has failed to result in any evident benefit, and a marked excess may be definitely deleterious. Too much iron in the diet interferes with phosphorus absorption by forming an insoluble phosphate and rickets may thus result on a diet otherwise adequate. As the Wisconsin workers have pointed out, this is an excellent example of the danger of following in nutrition the idea that "if some is good, more is better."

#### IODINE

The mature animal body is estimated to contain less than 0.00004 per cent of iodine, but, if this minute amount is not

<sup>1</sup> ELVEHJEM, C. A., E. B. HART, and W. C. SHERMAN, The availability of iron from different sources for hemoglobin formation, *J. Biol. Chem.*, **103**, 61-70, 1933; SHERMAN, W. C., C. A. ELVEHJEM, and E. B. HART, Further studies on the availability of iron in biological materials, *ibid.*, **107**, 383-394, 1934.

maintained through the food, disaster results. More than half of this iodine is in the thyroid gland, and it is in connection with the functioning of this gland that the body's need for iodine occurs.

**124. The Thyroid Gland.**—This gland consists of two parts lying on each side of the trachea at its upper end. In the case of an adult man it weighs about 1 oz. It produces an internal secretion which contains the hormone, thyroxine, isolated by Kendall in 1914 as a crystalline product containing about 65 per cent of iodine. The later work of Harrington established its structure as an iodine-containing amino acid (Sec. 79). Thyroxine is present as thyroglobulin, a conjugated protein. More recently Foster isolated diiodotyrosine (Sec. 79) from the gland. The removal of the thyroid early in life results in all species in a stunting of physical, mental, and sexual development. In adult animals the hair and skin show premature aging and mental and physical sluggishness may develop. In all cases there is a lowered basal metabolism (Sec. 222). It is probable that the primary function of the thyroid gland is to control the metabolic rate through the output of its hormone and that the more evident effects of thyroid deficiency are a result of a failure of this control. Most of the iodine that occurs in the tissues and fluids of the body other than the thyroid is probably in thyroxine serving its function in the control of metabolism.

Goiter is an enlargement of the thyroid gland. Medical men recognize two types: simple or endemic goiter which is caused primarily by a lack of iodine, and exophthalmic goiter which involves other structures of the body besides the thyroid gland. Simple goiter is much the more common type, and it is the one with which we are concerned in nutrition. It develops as a result of a failure of the thyroid tissue to supply enough secretion, owing either to a reduced supply of iodine for its manufacture or to an increased demand for the secretion by the body. It is a compensatory hypertrophy, that is an enlargement involving the formation of more tissue in an effort to supply more secretion. The demand for thyroxine varies in accordance with the activity of the body functions it controls, and thus, given a fairly constant supply of iodine in the diet, simple goiter is most likely to develop during periods of greatly increased need. In the human these critical periods are pregnancy and puberty. In farm animals, however, goiter usually shows itself in the young at birth as a

result of a deficiency of iodine in the rations of the mother during gestation. The young thus affected are born weak or dead (Fig. 13). On a deficient diet the mother is not able to supply the fetus with enough iodine. The danger is thus increased in the case of multiple births.

In calves, lambs, and kids the enlargement of the gland is very evident in the newborn. In pigs the most outstanding symptom of the deficiency is hairlessness. They are bloated and have thick skins and puffy necks. In foals the only symptom may be



FIG. 13.—Goiter in a new-born lamb. (*Courtesy of C. C. Culbertson of the Iowa Experiment Station.*)

extreme weakness at birth, resulting in an inability to stand and suck. A limited amount of data indicates that navel ill in foals may be lessened by feeding iodine to brood mares, but further evidence is required. Birds as well as mammals have enlarged thyroids as a result of iodine deficiency. Animals born alive with a well-developed goitrous condition usually fail to survive or remain weaklings. No treatment has been found particularly effective. Studies of goiter troubles in humans have clearly established that, while iodine is effective as a preventive, it may be harmful rather than beneficial as a treatment after the goiter has developed. While the nutritionist is concerned with prevention, treatment belongs entirely to the field of medicine.



Though a lack of iodine is the primary cause of simple goiter, it is recognized that other factors may contribute, notably the high-calcium content of the water in many goitrous regions. Infections from water supplies may also play a role, and there is some evidence that certain foods, notably cabbage, may contain a specific goitrogenic substance. It is clear, however, that any influence of these various factors on the occurrence of simple goiter is slight compared to iodine deficiency.

**125. Iodine Deficiency an Area Problem.**—The need for additional iodine in the rations of farm animals, as well as of humans, exists primarily in certain areas where the soil and thus the water and food crops are low in this element. There are various regions throughout the world where goiter troubles of varying degrees are very common in all species unless additional iodine is fed, and there are others where the trouble is entirely unknown. In the United States, the goiter areas are primarily in the Northwest and in the Great Lake region. It is estimated that, before iodine feeding was practiced in Montana, goiter caused an annual loss of many thousand pigs. Records from other areas show that serious losses in the sheep and cattle industries occurred which were largely prevented following the discovery of the lack of iodine as the causative factor. There are borderline regions in which goiter occurs only occasionally. When the usual iodine intake is little above the minimum requirement, an enlarged physiological demand by an individual may be responsible for the occasional troubles. A barely sufficient intake may be changed to an inadequate one by a change in the make-up of the ration.

Hundreds of years before iodine was discovered, people living in goitrous areas learned the usefulness of certain products, now known to be rich in iodine, as a preventive of goiter. The value of sea salt in comparison with certain inland deposits was early recognized. Our real understanding of the problem is very recent as indicated by the fact that the discovery by Baumann of iodine in the thyroid gland was not made until 1896.

**126. Iodine Requirements.**—It cannot be said that we have specific knowledge of the iodine requirements of the various species. This question has been studied in rats by noting the amount needed to maintain what is considered to be a normal content in the moisture-free thyroid gland, and also by determining the amount required to prevent any enlargement of the



gland. From such measures Levine, Remington, and von Kolnitz<sup>1</sup> conclude that the rat requires 20 to 40 micrograms of iodine per 1000 Cal. of food. This would mean an intake of 60 to 120 micrograms for a man eating 3000 Cal. Estimates from other methods have placed the adult human requirement as 30 to 70 micrograms, and higher for children. The amounts which have been found adequate, as supplements to the commonly fed rations, to prevent goiter in farm animals in areas where it otherwise occurs regularly are discussed later (Sec. 287).

While, in the case of farm animals, the principal demand for iodine occurs during pregnancy, it must be recognized that the thyroid gland must continue to function throughout life, and one might expect, therefore, that, in areas of very severe iodine deficiency, the continued feeding of an additional supply might be desirable. There is some positive evidence from certain areas in Central Europe that additional iodine improves growth and milk production and lessens certain reproductive trouble in various species of farm animals. The evidence is not entirely convincing, and there is negative evidence also. In the United States studies in the goiter belt have repeatedly failed to show benefit from the addition of iodine except for the specific purpose of the prevention of goiter. Recommendations for year-round feeding are usually put on an insurance basis or are suggested as the simplest way of making certain that the needs of breeding stock are taken care of in areas where goiter is very common. In an extensive experiment as to the value of iodine for livestock in central Pennsylvania, Forbes and coworkers<sup>2</sup> found no beneficial effects on the growth of dairy calves, lambs, pigs, or chickens, or on the incidence of abortion in dairy cattle or on egg production in pullets.

**127. Iodine Supplements.**—Where the occurrence of goiter shows that attention to iodine nutrition is needed, the most practical method is the use of some special source such as iodized salt or sodium or potassium iodide. None of the common feed

<sup>1</sup> LEVINE, H., R. E. REMINGTON, and H. VON KOLNITZ, Studies on the relation of diet to goiter. II. The iodine requirement of the rat, *J. Nutrition*, **6**, 347-354, 1933.

<sup>2</sup> FORBES, E. B., G. M. KARNS, S. I. BECHDEL, P. S. WILLIAMS, T. B. KEITH, E. W. CALLENBACH, and R. R. MURPHY, The value of iodine for livestock in central Pennsylvania, *J. Agr. Research*, **45**, 111-128, 1932.

stuffs, with the exception of fish meal made from saltwater fish can be relied upon to be rich in iodine. Dried kelp, a sea plant, is rich in the element and so is cod-liver oil. While special advantages are sometimes claimed for organic sources of iodine, the preponderance of the evidence indicates that the cheaper inorganic iodides are equally satisfactory. Certainly they have been proven effective. Free iodine and iodides are readily absorbed from the digestive tract and circulate in all body fluids. When massive doses of iodine are given as a therapeutic agent, an organic source has the advantage of slower absorption and less risk of harm from overdosage, but, in the amounts needed to prevent goiter, this is not a factor.

Any required addition of iodine need not be supplied every day, because the thyroid has a considerable capacity to store the element. This is illustrated by the fact that goiter has been prevented in children in goitrous areas by feeding sodium iodide periodically for a month and repeating twice yearly.

**128. Iodized Milk and Iodized Eggs.**—Large intakes of iodine by the dairy cow result in an increased content in the milk, and the same is true for the hen in egg production. It has been recently proposed that special sources of iodine should be fed to cows and hens in order to produce iodized milk and eggs for human consumption for the prevention of goiter. This is not a new idea for the textbook of Gorup-Besanez published in 1878 records the feeding of iodine to llamas in South America and the use of the iodine-containing milk as medicine. Such a practice does not seem desirable today from either the human or animal standpoint. The iodine secreted in milk and eggs is no more effective than iodized salt. Only a small and variable percentage of the iodine fed to the animal appears in the product, and thus this method of providing it to those who need it, even if honestly carried out, is more expensive and much less certain as regards the amount supplied, than is direct administration. The Committee on Foods and Nutrition of the American Medical Association has stated that the fortification of foods other than salt with iodine is unnecessary and may endanger public health. As far as the animals are concerned, the continuous feeding of the rather large intakes of iodine required to result in a substantial enrichment in their products is a practice which may result in definite harm, for the danger of overdosage is a real one.

### SULFUR

The body contains approximately 0.15 per cent of sulfur. This element occurs almost entirely in organic compounds, notably in proteins in which it is present as the sulfur-containing amino acids, cystine and methionine. Wool contains approximately 4 per cent of sulfur. The element occurs also in a number of special compounds such as glutathione (Sec. 84), insulin (Sec. 48), ergothionene, taurine, mercapturic acids, and others. All of these compounds are doubtless derived from proteins or the sulfur-containing amino acids. This fact indicates that meeting the sulfur needs of the body is primarily a matter of amino-acid nutrition. Since hair and wool are rich in sulfur-containing proteins, the influence of cystine and other sulfur compounds, as supplements to commonly fed rations, has been studied from the standpoint of the growth of these epidermal tissues, with generally negative results (Sec. 264). Free sulfur or inorganic sulfate in the diet has little if any value in nutrition, and excessive intakes of the free form are definitely toxic. The blood contains small amounts of sulfates. Thiocyanate ions are also present in blood, as well as in saliva and other secretions.

Both the feces and urine are paths of sulfur excretion. In the urine three forms occur: inorganic sulfates, the principal fraction, which represent the final stage of oxidation of organic sulfur; ethereal sulfur which is present in complex detoxication products; and neutral sulfur which occurs as cystine, taurine, thiosulfates, and other compounds. Since excreted sulfur arises primarily from protein catabolism, there is a rather constant ratio between it and the nitrogen in the urine. There is evidence that the excretion of neutral sulfur is proportional to the basal metabolism.

### MANGANESE AND ZINC

Manganese and zinc complete the list of elements which have definitely proven to be essential. Though this proof exists only for laboratory animals, it is probable that larger animals need them also.

**129. Manganese.**—Studies with rats, mice, and dogs have shown that manganese is an essential element for several body functions. It is particularly concerned in the physiology of reproduction. On a manganese-low diet, sexual maturity is delayed and ovulation is irregular. If conception occurs the

young are born weak or dead and their bodies contain less than half as much manganese as those born from mothers normally fed. In the male a diet devoid of the element causes a degeneration of the germinal epithelium. Growth is subnormal on a manganese-low diet. While certain studies have indicated that the element has a supplementary effect, as an addition to iron and copper, on hemoglobin regeneration in nutritional anemia, most workers have not been able to confirm this view, and it has been largely discarded. Wilgus and associates<sup>1</sup> have recently produced evidence that a lack of manganese is a cause of perosis, or "slipped tendon," an anatomical deformity of the tibial-metatarsal joint of young chickens.

The manganese content of the body is very small. The principal store is in the liver, but the element is also present in appreciable amounts in the pancreas, kidneys, sexual organs, skin, muscle, and bones. Its content in the body can be greatly increased by feeding. Excretion occurs principally through the feces.

So far as is known, the small amount of manganese needed is never deficient in the usual rations. Everson and Daniels<sup>2</sup> suggest a requirement of 0.2 to 0.3 mg. per kilogram for children. Peterson and Skinner<sup>3</sup> report data on the manganese content of a wide variety of foods. Seeds and leafy materials are relatively rich sources of this element.

**130. Zinc.**—Though the total amount is very small, zinc is rather widely distributed in the various tissues of the body, notably in the bones, hair, liver, pancreas, kidney, and muscles. The early studies of Bertrand, indicating that this element plays an essential role, have been recently confirmed and extended by others in experiments with both rats and mice. On a nearly zinc-free diet growth is retarded; and the development of the fur of rats is interfered with according to Stirn, Elvehjem, and Hart.<sup>4</sup>

<sup>1</sup> WILGUS, H. S., JR., L. C. NORRIS, and G. F. HEUSER, The role of certain inorganic elements in the cause and prevention of perosis, *Science*, **84**, 252-253, 1936.

<sup>2</sup> EVERSON, G. J., and A. L. DANIELS, A study of manganese retentions in children, *J. Nutrition*, **8**, 497-502, 1934.

<sup>3</sup> PETERSON, W. H., and J. T. SKINNER, Distribution of manganese in foods, *J. Nutrition*, **4**, 419-426, 1931.

<sup>4</sup> STIRN, F. E., C. A. ELVEHJEM, and E. B. HART, The indispensability of zinc in the nutrition of the rat, *J. Biol. Chem.*, **109**, 347-359, 1935.

Continued feeding of relatively large intakes of zinc results in a markedly increased store of the element in the body which store gradually disappears after the feeding is stopped. The main path of excretion is the feces. Zinc has been found in both human and cow's milk, and colostrum has been reported much richer than milk itself.

Zinc is widely distributed in the vegetable kingdom. The amounts present in the common foods of animals and man are greatly in excess of any probable need by the body, and thus, in practice, the question of a zinc requirement calls for no consideration. Neither does there seem to be any danger of harmful intakes. At least, studies have found no deleterious effects on growth, health, or reproduction in laboratory animals from the continued feeding of large doses.

#### ALUMINUM AND SILICON

**131. Aluminum.**—All plants and animals contain traces of aluminum. Thus far no evidence has been produced that it is an essential constituent of body tissues, and its presence is explainable on the basis that it is passively taken up and retained owing to its universal presence in foods.

In view of the widespread use of aluminum cooking utensils, much recent research has been devoted to possible toxic effects of the element. Much higher levels than occur in foods or waters have been continuously fed to rats, dogs, pigs, and man without observable harm. Since toxic effects have been reported from the injection of aluminum salts, it seems probable that the lack of harmful effect from rather large intakes in the food is due to a low degree of absorption. It is clear, however, that aluminum may interfere with the absorption of phosphorus. Intakes which are large enough to combine with a substantial part of the phosphorus of the diet render the latter unabsorbable and result in a lowering of the plasma phosphate, a decrease in bone ash, and rickets. Such an effect is not to be feared from natural foods, since they contain, at most, only a few parts per million of the element.

**132. Silicon.**—All animal tissues which have been examined have been found to contain silicon in varying amounts. It is present in the blood serum of farm animals as 1 to 2 mg. per 100 cc. No one has been able to demonstrate that the element plays

any essential role in the body, and thus its occurrence is generally regarded merely as an accumulation resulting from its universal occurrence in foods, particularly those of vegetable origin. It is interesting to note that silicon has been found in fetuses, although the significance of this finding is unknown. Relatively large amounts are found in lung tissue but this represents inspired rather than ingested material.

Mature roughages are especially rich in silicon since their cellulose is infiltrated with the element. Samples of timothy hay have been reported to contain as much as 0.7 per cent. The mineral metabolism studies of Forbes and Beegle<sup>1</sup> revealed an extensive metabolism of silicon in the dairy cow. In many instances the intakes equaled those of sodium, chlorine, and phosphorus. Most of the ingested element was recovered in the feces, but the balance studies recorded some storage in almost all cases, frequently 3 to 5 g. daily. Only very small amounts were found in the urine, the feces being by far the principal channel of excretion. In view of the fact that herbivorous animals normally ingest relatively large amounts of silica, it is interesting to note that the Cornell workers<sup>2</sup> have reared various species to maturity on synthetic diets of purified food materials which were certainly very low in the element. It has been reported that silica constitutes up to 77 per cent of the ash of feathers and that it probably performs a useful function in maintaining their rigidity.

### FLUORINE

While fluorine is found in various parts of the body, notably in the hair, its regular occurrence in the teeth and bones to the extent of 0.02 to 0.05 per cent is of primary interest to the nutritionist. The element is an integral part of the structure of the bones and teeth, which suggests that it is an essential constituent. This fluorine content, however, is subject to wide variations according to the amount of the element present in the diet. Bones containing more than 1 per cent have been produced by adding fluorine salts to the diet of experimental animals.

<sup>1</sup> FORBES, E. B., and F. M. BEEGLE, The mineral metabolism of the milch cow, *Ohio Agr. Expt. Sta. Bull.* 295, 1916.

<sup>2</sup> MADSEN, L. L., C. M. McCAY, and L. A. MAYNARD, Synthetic diets for herbivora, with special reference to the toxicity of cod-liver oil, *Cornell Mem.* 178, 1935.

While no one has been able to show that fluorine is an essential constituent of the bones and teeth, many studies have proved that the dietary levels which greatly increase the fluorine content of these structures do result in definite harm. These experimental observations throw doubt on the belief that the element is essential, and furnish some support for the view of others that it is purely a waste product. Since fluorine is rather widely distributed in common foods and since its content in certain mineral

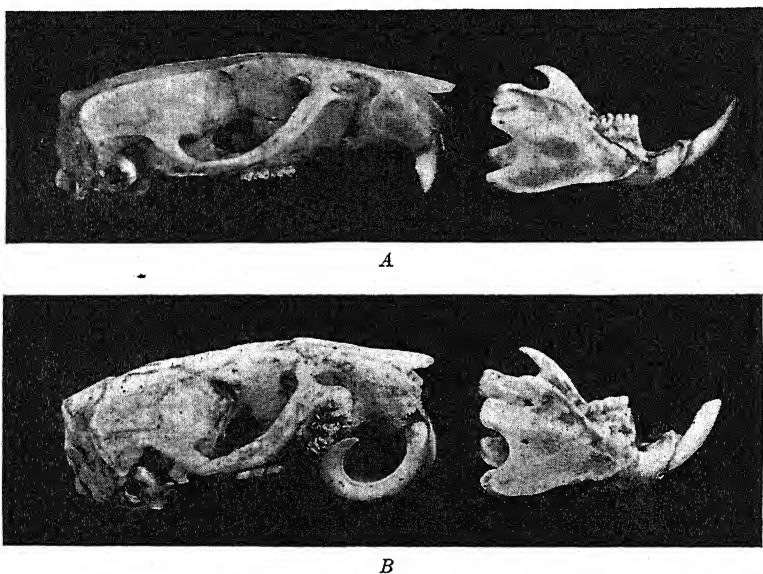


FIG. 14.—Skulls of rats fed on diets with and without fluorine. A, normal skull. B, thickened skull with elongated and chalky upper incisors caused by excessive fluorine intake.

supplements and in the water of various areas is dangerously high, the preceding observations also indicate why, from the standpoint of nutrition, fluorine requires attention as a harmful element rather than as an essential one.

**133. The Toxicity of Fluorine.**—While it has long been known that the ingestion of certain doses of fluorides is fatal to man and animals, it has been learned only very recently that rations containing small fractions of a per cent are injurious, particularly if continuously fed. The most evident effect of low intakes of fluorine is on the bones and teeth.



The bones lose their normal color and luster, become thickened and softened, and the breaking strength is decreased. Bony outgrowths from the surface, called exostoses, occur. The fluorine content of the bone increases many times, and its magnesium content also increases but there is a decrease in carbonate. The total ash content is lowered by high levels of intake. There are also characteristic histological changes. The effects upon the teeth are similar though they manifest themselves somewhat differently, particularly in certain species. In the rat the enamel loses its glistening yellow color and becomes chalky and brittle. The permanently growing incisors do not wear away normally and either the upper or lower incisors become elongated. These changes are illustrated in Fig. 14, which also shows the thickening of the skull bones resulting from fluorine feeding. In hogs and cattle, defects in the enamel are produced and the teeth become soft and worn down until in some cases the pulp cavities are exposed. The teeth become sensitive to cold water and food consumption is interfered with.

In children an excessive fluorine intake is responsible for the development of mottled enamel. This condition is characterized by the presence of chalky-white patches on the surface of the teeth. Frequently the entire tooth surface is dull white in color and the enamel becomes pitted and may chip off. Secondly the teeth may become stained showing a coloration which varies from yellow to black. Mottled teeth are structurally weak owing to an interference with the normal development of the enamel. Mottling is chiefly a defect of the permanent teeth which results during their formation. Normally formed teeth do not become mottled later. In the permanently growing teeth of the rat, however, bleaching may be produced at any time during life. While mottled enamel has long been known to occur in various parts of the world, it was not until 1931, as a result of the work of Smith and coworkers,<sup>1</sup> that the defect was shown to be caused by fluorine. These investigators found that an excessive fluorine content in the water was responsible. The fact that mottled enamel will occur in children who regularly drink water containing as little as 1 p.p.m. of fluorine illustrates how small an amount may cause injury. Mottled enamel has also been

<sup>1</sup> SMITH, M. C., E. M. LANTZ, and H. V. SMITH, The cause of mottled enamel, a defect of human teeth, *Ariz. Agr. Expt. Sta. Tech. Bull.* 32, 1931.



reported to occur in cattle in areas where the water is high in fluorine.

While injuries to the bones and teeth are the initial or the most evident effects of fluorine, higher intakes or long continued feeding interfere with food consumption, growth, and milk production. How fluorine produces these various end results is not known. The primary effect in so far as the bones and teeth are concerned does not seem to be upon calcium and phosphorus metabolism. The element has a deleterious effect upon various enzymatic actions of metabolism, and it is possible that this is the basis of the more evident injuries. Of course, the wearing down of the teeth interferes with food consumption and this in turn with growth and production.

**134. Variations in Fluorine Toxicity.**—It is evident that the toxic level of fluorine varies with the species and that it is also dependent somewhat on the form in which the fluorine is ingested. The amount in drinking water which causes mottled enamel in children represents an intake of 1 to 2 mg. per day. No such low level has been reported injurious to farm animals. Extensive experiments by Phillips, Hart, and Bohstedt<sup>1</sup> suggest that the critical level of tolerance for continuous feeding to dairy animals is 2 to 3 mg. per kilogram of body weight. An intake of fluorine as rock phosphate which corresponded to approximately 0.008 per cent of the total dry matter proved definitely harmful to the teeth, bones, and general health. The cows declined in milk during the fourth and fifth years of feeding. Earlier deleterious effects on milk yield were noted from somewhat higher levels. Failure to come in heat regularly was also observed. Rations containing approximately 0.03 per cent of fluorine decrease feed consumption in pigs and injure their bones. Considerably higher levels are required to cause injury in chickens. Rat growth is retarded by a level of 0.02 per cent, but their teeth show bleaching from as low a level as 0.0015 per cent.

It cannot be considered that the levels here cited represent the lowest ones which may cause injury, particularly in the long-time feeding of breeding and lactating animals since fluorine is cumulatively harmful. Definite physiological disturbances may

<sup>1</sup> PHILLIPS, P. H., E. B. HART, and G. BOHSTEDT, Chronic toxicosis in dairy cows due to the ingestion of fluorine, *Wis. Agr. Expt. Sta. Research Bull.* 123, 1934.

take place at levels which show no effects in terms of growth or evident bone and teeth injuries. The Wisconsin workers point out that the tolerance limit which is safe for cattle lies well below the levels used in their experiments.

Experiments with rats, pigs, and chickens have reported variations in toxicity according to the form in which the fluorine is ingested. Sodium fluoride has been found a much more toxic source than rock phosphate, calcium fluoride, or other insoluble compounds. The differences have been measured in terms of growth and marked bone injury. At the very low levels which cause the bleaching of the enamel of rats' teeth, however, a large number of compounds, soluble and insoluble, were found equally effective per unit of fluorine by Smith and Leverton.<sup>1</sup> This finding has been confirmed by Ellis and Maynard for sodium fluoride and bone meal, using both the bleaching effect and increase in fluorine content of the bones and teeth as measures.

A recent preliminary study by Sharpless<sup>2</sup> has shown that the addition to the diet of several times as much aluminum as fluorine markedly lessens the harmful effect of excessive fluorine intake on growth and enamel formation in rats. The protective effect is considered to be due to the formation of an insoluble, and thus unabsorbable compound, of the two elements in the intestine. Further data should be watched for with interest. The feeding of large intakes of aluminum to counteract fluorine would appear impracticable because of its similar effect in rendering phosphates unabsorbable, unless the fluorine compound is formed by preference.

**135. Practical Aspects of Fluorine Toxicity.**—Aside from the special situation in areas where the water is high in fluorine, our practical interest in this fluorine problem from the standpoint of farm animals lies in the fact that certain mineral supplements may contain excessive amounts. Rock phosphate has been found harmful on this basis (Sec. 134), and certain products made from rock, such as acid phosphate and dicalcium phosphate, contain significant amounts of fluorine unless the manufacturing process has included a special treatment to remove most of it. It is

<sup>1</sup> SMITH, M. C., and R. M. LEVERTON, Comparative toxicity of fluorine compounds, *Ind. Eng. Chem.*, **26**, 791-797, 1934.

<sup>2</sup> SHARPLESS, G. R., Limitation of fluorine toxicosis in the rat with aluminum chloride, *Proc. Soc. Exptl. Biol. Med.*, **34**, 562-564, 1936.

believed that any mineral supplement which is used in liberal amounts for continuous feeding should not contain more than 0.1 per cent of fluorine. This figure may be unnecessarily low, but this level would appear to allow a large margin of safety as regards the intakes which have proven harmful, and such a margin is wise, at least pending further research. The level would not exclude the bone meals or dicalcium phosphate made from bones, since these products normally contain less than 0.05 per cent. Bone meal has been repeatedly used in control rations where various fluorine compounds have been under study and no one has reported fluorine injury to farm animals from the bone itself.

While fluorine occurs in most of the common feed stuffs, in general, the level does not exceed 1 or 2 p.p.m. Considerably higher values have been reported for certain fish meals. The question as to whether phosphate manuring constitutes a hazard by reason of an increased fluorine content in the crops so fertilized has been answered in the negative by Hart and coworkers.<sup>1</sup> These workers did find, however, a high-fluorine content in the drainage water from plots treated with rock phosphate, which raises the question as to whether long continued manuring with products high in fluorine may increase this element in the drinking waters to dangerous levels. The use of fluorine insecticides appears to be hazardous in so far as human nutrition is concerned and a similar problem may be created by the use of these insecticides on crops eaten by animals.

The data in the literature as to the fluorine content of various products vary widely, and the same is true for certain animal tissues. Recently obtained values may be considered the more reliable because the methods of analysis have been improved, but limitations in this respect are still recognized. It is possible that the further refinement of methods may modify some of the data upon which our present ideas are based.

#### SELENIUM

Selenium has very recently been found responsible for a peculiar disease of livestock which has long been known to exist in certain

<sup>1</sup> HART, E. B., P. H. PHILLIPS, and G. BOHSTEDT, Relation of soil fertilization with superphosphates and rock phosphate to fluorine content of plants and drainage waters, *Am. J. Pub. Health*, **24**, 936-940, 1934.

regions of the world. In the United States, it occurs in some areas of the plains region, notably South Dakota, and is known locally as "alkali disease" or "blind staggers." In chronic cases there is a loss of hair from the mane and tail in horses and from the tail in cattle and a general loss of hair in swine. The hoofs slough off, lameness occurs, food consumption decreases, and death may occur by starvation. These external symptoms are accompanied by marked pathological changes which are revealed on post mortem.

Franke, chemist of the South Dakota Experiment Station, traced the cause of the disease to crops grown in localized soil areas. This finding led to a cooperative study with the U. S. Department of Agriculture which resulted in the discovery of selenium as the specific factor. Various food crops may contain toxic levels of the element as a result of the amounts present in the soil in the areas in question. The soil may contain as much as 40 p.p.m., but any soil which contains more than 0.5 p.p.m. is potentially dangerous. Both the forage and the grains contain toxic levels. Different plants vary greatly in the amounts they take up, but the concentration in the plant is generally much greater than in the soil. On a soil containing 9 p.p.m. of selenium certain crops have been found to contain as much as 1200 p.p.m. Chronic toxicity is caused by rations containing as little as 8.5 p.p.m. of selenium. Acute cases of poisoning have been reported from levels of 500 to 1000 p.p.m. Young animals are especially susceptible and growth is retarded with levels too low to cause other evident symptoms. This selenium injury is not limited to animals, for human cases have also been reported.

No method has been found of counteracting this selenium injury, either by dietary means or by soil treatment. In areas where it occurs, toxic levels in the food crops are so general that there is no safe food supply. There seems to be no solution other than to abandon the area. Certainly a livestock industry cannot flourish, and the production of food crops constitutes a menace to both animals and man. The milk and eggs from cows and hens fed rations containing selenium contain the element.

Rats are susceptible to this selenium poisoning, and thus they have been used as test animals to study the physiological effects of the element and to correlate the injury with the selenium content of the plants and soils. Our specific knowledge of these aspects

is due particularly to the work of Franke and associates.<sup>1</sup> They have also studied selenium poisoning with chickens.

An excellent statement of the history of the selenium problem, the results of research to date, and their implications in agriculture has been presented by Trelease and Martin.<sup>2</sup> It is evident that the problem needs much further study, and it is possible that selenium is not the sole factor involved in these area troubles.

### OTHER MINERALS

*Nickel and cobalt* have been identified in various tissues by Bertrand and others, but, in some very recent investigations, failure to find the minerals has been reported. Clearly they can be present in only very small amounts. No one has shown that nickel is essential for body function. Underwood and Filmer in Australia have obtained results suggesting that a disease of sheep and cattle characterized by a progressive emaciation and anemia is due to a deficiency of cobalt. Both cobalt and nickel have been reported to activate arginase.

*Arsenic* is found in small amounts in the skin, hair, hoofs, thyroid gland, and elsewhere in the body. The question is still open as to whether the element is necessary for the functioning of living cells or whether its presence in the body is due merely to its accumulation from the traces which occur in many foods. The harmful effects of small intakes, which create practical problems notably in connection with insecticides, are well understood. Certain mineral supplements prepared from rock phosphate may contain an excessive amount of arsenic unless special methods are used to eliminate it.

*Bromine* is found in the blood and in various glands of the body. It has been reported that the pituitary gland secretes a bromine-containing hormone, but recent work has not been able to confirm this. There is no certain evidence that the element plays any essential role in the body, and its presence may be entirely fortuitous owing to its widespread occurrence in small amounts in food.

<sup>1</sup> Franke and his coworkers have published a long series of papers in the *Journal of Nutrition* during the period 1934-1936.

<sup>2</sup> TRELEASE, SAM F., and ALAN L. MARTIN, Plants made poisonous by selenium absorbed from the soil, *Bot. Rev.*, **2**, 373-396, 1936.

*Boron* is essential for plant growth and has been reported present in the animal body, but there is no proof that it has any function in the latter.

### SOME PHYSIOLOGICAL EFFECTS OF MINERALS AS A GROUP

The previous discussions have shown that with certain mineral elements their quantitative relationships to each other are important as well as their absolute amounts in the diet. There are physiological effects resulting from the relations among the minerals as a group, or from their intakes as a whole, which also require brief consideration.

**136. Acid-base Balance in the Ration.**—It has been noted that the pH of the blood and other body fluids must be held in a very narrow range for health and normal function. In contrast, the food ingested may exhibit wide ranges of potential acidity or alkalinity depending upon the amounts of the various mineral elements present. Thus the question of the influence of the acid-base balance of the diet upon metabolism has received much study. This balance is determined by calculating the equivalent in normal acid of the acid-forming minerals, chlorine, phosphorus, and sulfur, and figuring the equivalent in normal alkali of the base-forming elements, sodium, potassium, calcium, and magnesium. Calculated in this way the potential acidity of wheat exceeds its potential alkalinity by the equivalent of 12 cc. of normal acid per 100 g. It is an acid-forming food. On the other hand, alfalfa has a net potential alkalinity of 93 cc. and is, therefore, a base-forming food. In general, seeds and their by-products are acid forming, while roughages are base forming. High-protein diets tend to be acid forming owing especially to the sulfur present. The acid-base balance of a ration can be calculated from the figures for its ingredients.

In figuring acid-base balance, no account is taken of organic acids. Citrus fruits contain considerable amounts of these acids and yet they are base-forming foods. Organic acids are not taken into consideration, because, for the most part, they are oxidized or otherwise metabolized in the body and thus do not function as acids in the fluids. The extent to which they are oxidized is dependent upon the kind and amounts present in the diet. Large amounts of citric acid are readily oxidized, but this is less true for certain others.

It is recognized that highly acid-forming diets decrease the alkali reserve of the blood and may result in the excretion of fixed bases, even from the bones. The urine becomes more acid, and thus its solvent power for uric acid is decreased with a resulting increasing tendency to the formation of kidney stones. It has been shown, however, that the body is able to tolerate a moderate excess of acid-forming elements without harm. Despite much study, the question of the significance of the acid-base balance in human diets which are satisfactory in other respects appears to be still open. In farm animals most of the experiments in which this question has been studied by the ingestion of mineral acids have indicated that the animals have effective means of getting rid of the acid without harm. This has been shown for swine by the Iowa workers, Lamb and Evvard,<sup>1</sup> though Forbes and associates<sup>2</sup> earlier reported that the alkali reserve was decreased in this species by cereal feeding. The production of ammonia from urea provides a particularly effective agency for the neutralization of acid without increasing protein catabolism or loss of fixed base. The urine of Herbivora is generally alkaline, reflecting the fact that their ration is usually base forming. The urine of Carnivora is commonly acid, and that of Omnivora is either acid or alkaline depending on the nature of the diet. The feeding of large amounts of highly acid silage does not cause any serious physiological disturbance because the acidity is due to organic acids which for the most part are readily oxidized. According to recent work, silage which has been preserved with mineral acid by the *A.I.V. process* is well tolerated by cows, sheep, and pigs, at least when the acid is partially neutralized by lime.

**137. Saline and Alkaline Waters.**—The water supplies for animals in certain regions have such high concentrations of various salts as to interfere with growth, lactation, and reproduction.

<sup>1</sup> LAMB, A. R., and J. M. EVVARD, The acid-base balance in animal nutrition. I. The effect of certain organic and mineral acids on growth, well-being and reproduction of swine. II. Metabolism studies on the effect of certain organic and mineral acids on swine, *Iowa Agr. Expt. Sta. Research Bull.* 70, 1921.

<sup>2</sup> FORBES, E. B., J. O. HALVERSON, and J. A. SCHULZ, Alkali reserve of swine as affected by cereal feeding and mineral supplements, *J. Biol. Chem.*, **42**, 459-463, 1920.

This problem has been critically studied by Heller<sup>1</sup> and the studies have produced some very valuable data on the tolerance of animals to specific salts and to large intakes of minerals in general. The waters studied were heavily saturated with chlorides and sulfates of sodium, calcium, and magnesium and contained minor quantities of carbonates, bicarbonates, and other ions. It was found that the damage depended more on the total amount of salts present than on any specific one, thus representing an osmotic effect rather than an injury from any particular ion. The maximum concentration of soluble salts which could be safely tolerated appeared to lie between 1.5 and 1.7 per cent. Alkalies were more injurious than neutral salts. Chlorides were less harmful than sulfates, and organic salts less harmful than inorganic. Magnesium chloride was more injurious than the calcium or sodium salt. Sheep were more resistant to injury than cattle and cattle more than hogs. More recent studies by Heller have indicated that the cause of the injury is not due to any influence on the pH of the intestinal tract, digestibility, or nitrogen retention.

**138. Complex Mineral Mixtures.**—So far as is now known, except in very special cases, the only supplementary minerals that are ever needed in the commonly fed rations of farm animals, beyond the suckling stage, are salt, calcium, phosphorus, and iodine. There are many proprietary mineral mixtures on the market which contain a large number of other minerals such as potassium, magnesium, iron, sulfur, and copper. It is a waste to feed unneeded minerals and several cases of harmful effects of excessive intake of essential elements have been mentioned in previous discussions. It is both good economy and good nutrition to limit any intake of supplemental minerals to those which are actually deficient in the ration being fed. Commonly salt is the only supplement needed. The writer believes that complex mineral mixtures have no place in scientific feeding, particularly when they contain purgatives, worm remedies, and tonics and are thus sold as "shot-gun" prescriptions for all ills, nutritional and otherwise.

<sup>1</sup> HELLER, V. G., Saline and alkaline drinking waters, *J. Nutrition*, 5, 421-429, 1932; The effect of saline and alkaline waters on domestic animals, *Okla. Agr. Expt. Sta. Bull.* 217, 1933.



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## CHAPTER VIII

### THE VITAMINS

Over one hundred years ago Prout<sup>1</sup> stated that there were three great staminal or proximate principles—a saccharine principle, an oily principle, and an albuminous principle—which provided the essential nutritive constituents of all organized bodies. Until early in the present century these principles, which later became known as the carbohydrates, fats, and proteins, were considered to be adequate to meet all the nutritive needs of the body other than its mineral requirements. Then came the discovery that there were other organic dietary essentials, previously unrecognized because needed in only minute amounts, which were not supplied by the early known principles. These are the nutrients which we class as vitamins. Knowledge as to the chemical nature of these dietary essentials lagged far behind the discovery of their nutritional importance, and thus, in the absence of any chemical basis for classifying them, they were grouped together and the term *vitamine*, coined by Funk in 1912 to designate a single one which he believed at the time to be an amine, was taken over to cover the group. From a physiological and nutritional standpoint, there are many advantages in considering the vitamins as a group, but it should be borne in mind that they are unrelated chemically and that the group name has no chemical significance.

In the 25 years since the first vitamin was discovered, our knowledge in this field has greatly advanced. New ones have been reported from time to time, their physiological functions

<sup>1</sup> PROUT, WILLIAM, Chemistry, meterology and the function of digestion, Bridgewater Treatise, William Pickering, London, 1834. Prout (1785-1850), an English physician, was a profound student of the relations of chemistry to physiology. He discovered hydrochloric acid in the gastric juice, showed that the snake excretes its nitrogen as uric acid, and demonstrated that the developing chick takes calcium from the shell to build its bones.

have been worked out, and the chemical nature of several of them has been established. At the same time, the subject has become more complicated each year, not only because of the increasing body of information, but also because of the mass of conflicting or unconfirmed data which is characteristic of a rapidly developing, new field of knowledge. There are at least eight vitamins for which the information is sufficiently complete and definite that their existence is generally accepted. There are many others which have been proposed as the result of various experiments. The claims for the existence of some of the latter group are supported by a considerable amount of evidence, but, in most cases, the knowledge remains too fragmentary to be at all conclusive. It is highly unlikely that all of them are distinct essentials. On the other hand, the probability that there are still undiscovered vitamins must be recognized. A textbook on nutrition can deal only with those which are well established by a substantial amount of generally accepted evidence. For a knowledge of the status of the many others which have been proposed, the student must consult the voluminous literature in this field, and, as is true for an up-to-date knowledge of the subject of vitamins in general, he must follow the new contributions as they appear in the various journals. He will find this no easy task, and he will also come to realize that complete knowledge of the subject of the vitamins lies far in the future.

A generally recognized vitamin is one that has been proven an essential dietary constituent for one or more species. Some of them are certainly not needed by all species; none have been tested for all. The knowledge as to the needs of the various farm animals is especially fragmentary. Most of the information regarding vitamins has been obtained from experiments with rats, guinea pigs, pigeons, and chickens. These species differ markedly among themselves as to the ones essential for them, and further differences are recognized when the larger animals are considered. It is important to bear in mind at the outset that all vitamins are not required by all species and that the specific needs of each must be actually determined; they cannot be assumed from findings with other species. It should also be remembered that no single food is rich in all the vitamins. Each vitamin has a somewhat different distribution from the others, in terms of the materials which make up the food supply. Our knowledge here

must be specific even as is the case for the requirements for the different animals.

**139. Development of the Vitamin Concept.**—Though the incidence of some of them has doubtless increased in modern times owing to changes in dietary habits, the specific diseases, which we now know to be due to the absence of the recently discovered vitamins, date far back in history. Scurvy has been a scourge of various peoples at least since the time of Hippocrates (400 B.C.), while beriberi was apparently known to the Chinese hundreds of years earlier. Though the specific evidence is comparatively recent, it is clear that, by the trial-and-error method, various individuals and peoples gradually learned that certain of these troubles were associated in some way with the nature of the diet and that specific foods were helpful in their treatment. The very early use by the Chinese of substances rich in vitamin A as remedies for night blindness, now known to be caused by a lack of this vitamin, is evident from the recent studies of Mar and Read.<sup>1</sup> These workers found that 20 animal and 65 vegetable products listed as remedies for this blindness in the ancient Chinese Herbal contained the vitamin in much larger amounts than supplied by cod-liver oil. In 1747, Lind, a British naval surgeon, showed that the juice of citrus fruits was a cure for scurvy. Cod-liver oil was used as a specific for rickets long before anything was known about the cause of this disease.

During the nineteenth century, many isolated observations were made which gradually led up to the discovery of vitamins as the causes of these disorders now called deficiency diseases. Prior to 1816, Magendie<sup>2</sup> observed in a dog what was undoubtedly xerophthalmia, in an experiment constituting a forerunner of the purified-diet method which was responsible 100 years later for the discovery of vitamin A. In the latter part of the century, several men made observations which led them to suggest that there were other dietary essentials besides the early recognized proximate principles, but these unorthodox suggestions at first received little attention. In 1881, Lunin reported studies made in Bunge's laboratory showing that mice would not grow on an

<sup>1</sup> MAR, PETER G., and B. E. READ, Chemical examination of Chinese remedies for night blindness, *Chinese J. Physiol.*, **10**, 273-284, 1936.

<sup>2</sup> MAGENDIE, FRANÇOIS, Sur les propriétés nutritives des substances qui ne contiennent pas d'azote, *Ann. chim. phys.*, **3**, 66-77, 1816.

artificial mixture made up of the proximate principles of milk. He expressed the view that there might be "unknown substances" essential for life in addition to proteins, fats, carbohydrates, and salts.

During the last decade of the century, Eijkmann, a physician working in the Dutch East Indies, was led to study polyneuritis in birds in view of its similarity to beriberi in man. He found that the disease was caused by an exclusive diet of polished rice and cured by adding the polishings. He also noted that beriberi in prisoners eating polished rice tended to disappear when a less highly milled product was fed. In these various studies, published in 1897, he was clearly dealing with the factor which later became known as vitamin B. The studies of Eijkmann were extended by Grijns, another Dutch scientist who made important contributions to the early knowledge of vitamins. Prior to the work of Eijkmann, Takaki, director-general of the Japanese Navy, sent two ships in 1887 on a nine-month voyage to test the effect of dietary additions on the incidence of beriberi. Of the crew which received mostly polished rice and dried fish, 60 per cent developed the disease, while in the other ship where this diet was supplemented with more meat, vegetables, and milk only 14 cases occurred among the 276 men. At that time the beneficial effect was erroneously ascribed to the larger amount of protein in the diet.

With the opening of the twentieth century, the earlier work of Lunin with artificial diets was repeated by others, notably by Pekelharing of the University of Utrecht who was familiar with the course of the studies in the Dutch East Indies and by Hopkins in England. Again it was concluded that the proximate principles would not suffice. As stated by Hopkins in 1906: "No animal can live on a mixture of pure protein, fat, and carbohydrate, and, even when the necessary inorganic material is carefully supplied, the animal still cannot flourish. The animal body is adjusted to live either upon plant tissues or other animals, and these contain countless substances other than protein, carbohydrate and fats." He coined the term *accessory food factors* for these substances.

From 1907 to 1913, Holst and Frölich of the University of Christiania carried out their classic investigation on scurvy. They were directed to investigate scurvy in Norwegian sailors.

They showed that guinea pigs developed this disease on cereals and bread and that it was cured by small amounts of fresh cabbage or carrots. They concluded that scurvy was due to the absence of a specific chemical factor, which was destroyed by cooking. They demonstrated a relationship between human and guinea pig scurvy.

Following work published in 1909 by Stepp showing the necessity of some constituent contained in the lipid fraction of certain natural foods, definite proof of the existence and specific physiological function of vitamin A was furnished in 1913 by the independent investigations of McCollum and Davis<sup>1</sup> and of Osborne and Mendel.<sup>2</sup> This specific knowledge resulted from carefully controlled experiments by the purified-diet method (Sec. 179). Dating from 1913, the extension of the knowledge of vitamins proceeded very rapidly.

In this brief statement of the historical background of the vitamin concept, only a few of the men concerned in its development have been mentioned. The discussion suffices to show that many scientists throughout the world contributed and that it is impossible to name any one person or group as the discoverer of this far-reaching concept. Such is the often-repeated story in scientific investigations. Links in the chain of facts are supplied and gradually put together by various workers over a period of years. Finally some one man may complete the chain, and a discovery is announced; but he may deserve no more, and sometimes even less, credit than others who made the previous observations which he used and extended in making the final contribution.

**140. Fat-soluble and Water-soluble Vitamins.**—McCollum proposed the names fat-soluble A for the factor found in butter and water-soluble B for the one concerned with beriberi as descriptive terms, since the first was extractable from foods with fat solvents and the second with water. On a similar basis, the antiscorbutic vitamin was later called water-soluble C. Though these descriptive adjectives were eventually given up they are still frequently used as general terms in classifying the vitamins.

<sup>1</sup> MCCOLLUM, E. V., and MARGUERITE DAVIS, Necessity of certain lipins in the diet during growth, *J. Biol. Chem.*, **15**, 167-175, 1913.

<sup>2</sup> OSBORNE, THOMAS B., and LAFAYETTE B. MENDEL, The influence of butter-fat on growth, *J. Biol. Chem.*, **16**, 423-437, 1913.



Thus the fat-soluble vitamins include A, D, and E, while B, C, and certain others are classed as water soluble.

In the present chapter, these various vitamins are discussed as regards their physiological effects, chemical nature and properties, and distribution in feeds. The specific requirements for growth, lactation, and other body functions are discussed in later chapters.

#### VITAMIN A

**141. Physiological Function.**—The initial identification of vitamin A as an essential nutritive factor by McCollum and Davis and by Osborne and Mendel was based on the observation that growth was inhibited in its absence and was resumed with its addition to the diet. Today, it is recognized that this effect is not a distinguishing feature of vitamin A, since most of the other vitamins are also essential for normal growth. Specific functions of the vitamin were soon uncovered. Osborne and Mendel quickly supplemented their initial observations by noting in the rat the production of *xerophthalmia*, an eye trouble known in children for many years. This is a disease characterized by a dry condition of the cornea and conjunctiva. In its development, the eyes become red, followed by cloudiness and ulceration which may terminate in blindness. If the injury has not proceeded too far, it may be cured by adding the vitamin to the diet. History shows that xerophthalmia has caused a very large amount of blindness in children, particularly among the poor or the more backward peoples. It is estimated that even today, in certain areas of the world where poverty is widespread and where the restricted diet is low in vitamin A, one out of five of the children shows symptoms of this eye disease.

The development of xerophthalmia represents a rather advanced stage of vitamin A deficiency. An earlier eye symptom in humans and certain animals, notably the cow, is *night blindness*. Here there is an inability to see in a dim light owing to an injury to the retina. In the chick, vitamin A deficiency causes the secretions of the tear glands to dry up. Infection may then occur resulting in a discharge which causes the lids to stick together. A condition which develops in the cow is shown in Fig. 15.

Wolbach and Howe<sup>1</sup> are responsible for discovering a more fundamental and generalized effect of the absence of the vitamin. They found that the normal epithelium in various locations throughout the body became replaced by a stratified, keratinizing epithelium. This effect has been noted in the respiratory, alimentary, and genitourinary tracts, as well as in the eye. This keratinization lowers the resistance of the epithelial tissues to the



FIG. 15.—Advanced state of eye lesion in vitamin A deficiency. (Courtesy of G. H. Hart, California Experimental Station.)

entrance of infective organisms. Thus respiratory troubles, such as colds and sinus infections, tend to be more severe in vitamin A deficiency. The same is true for certain gastrointestinal and kidney troubles. The formation of kidney and bladder stones is favored because the damaged epithelium interferes with the normal secretion and elimination of the urine. Xerophthalmia follows an injury to the epithelium of the eye. While a bacterial invasion has been frequently noted here, there is disagreement as to its being necessarily involved.

<sup>1</sup> WOLBACH, S. B., and P. R. HOWE, Tissue changes following deprivation of fat-soluble A vitamin, *J. Exptl. Med.*, **42**, 753-777, 1925; Vitamin A deficiency in the guinea-pig, *Arch. Path. Lab. Med.*, **5**, 239-253, 1928.

The recognition that certain infections were more common in vitamin A deficiency led to the proposal that it be called the anti-infective vitamin. Further studies have shown that this term is not justified. In large-scale, clinical experiments with children and adults, enriching their normal diets in vitamin A has failed to result in any sufficient decrease in colds and other respiratory troubles to indicate that the vitamin builds up immunity, although there was evidence of a decrease in the duration and severity of such troubles. Serological and bacteriological tests have failed to show convincingly that the defensive powers of the organism are strengthened by increased intakes of the vitamin. It would appear that a diet adequate in the vitamin is desirable to help maintain what may be considered the normal powers of resistance, but the early idea that additional intakes would largely obviate specific infections which enter through the epithelium has proved false. Aside from its curative effect on xerophthalmia, there is no evidence that the administration of the vitamin after an infection has become established will shorten its course or lessen its severity.

These observations do not mean that this keratinization of the epithelium is of little importance. There are many troubles, noninfective in character, which increase following its occurrence. This is particularly true of gastrointestinal and urinary troubles. There is a specific interference with reproduction caused by this altered epithelium which is of great importance, as is discussed later (Sec. 288). Keratinization is also responsible for certain skin troubles, such as dryness, occlusion of the hair follicles, and eruptions.

A lack of vitamin A results in the development of certain nervous disorders exemplified by a lack of coordination and spasms. Following some earlier suggestive observations by Hart and McCollum, Hughes, Aubel, and Lienhardt<sup>1</sup> definitely showed these symptoms in pigs to be caused by a deficiency in their ration and found that the symptoms were accompanied by a degeneration in the nervous system. Later the histopathology was worked out in more detail by others. There is a degeneration of the medullary sheath of peripheral nerves. The changes in rats have

<sup>1</sup> HUGHES, J. S., C. E. AUBEL, and H. F. LIENHARDT, The importance of vitamin A and vitamin C in the ration of swine, *Kan. Agr. Expt. Sta. Tech. Bull.* 23, 1928.

been thoroughly described by Zimmerman<sup>1</sup> and by Sutton, Setterfield, and Krauss.<sup>2</sup>

A lack of vitamin A is responsible for certain injuries to the teeth involving pathological changes in the odontoblasts and pulp. It also results in a loss of appetite which is doubtless one cause of the growth failure.

**142. Avitaminosis A in Various Species.**—Most of the controlled studies on vitamin A have been carried out with the rat, but it is known to be required by a large number of species including man, cattle, swine, chickens, pigeons, rabbits, monkeys, and others. Aside from a failure of growth, which is an inevitable result in all, the characteristic symptoms vary somewhat in different species. In cattle, night blindness is an early symptom, followed by lesions indicative of xerophthalmia, and complete blindness may result. There are characteristic nervous disturbances, respiratory troubles, and a reproductive failure simulating infectious abortion as is described later (Sec. 288). The syndrome in cattle has been very completely described by Guilbert and Hart.<sup>3</sup>

In swine, nervous troubles are an early and important symptom. There are stiffness, lack of coordination, and spasms. Eye lesions are seldom seen, though defective vision may occur. There are characteristic disturbances in reproduction, and the decreased resistance to infection shows itself in an increase in the incidence of pneumonia. In poultry, eye symptoms occur, and there are creamy-white pustules in the roof of the mouth and along the esophagus. Respiratory troubles and nervous disturbances have also been reported. The symptoms in sheep are apparently similar to those in cattle. In horses, according to Klemola,<sup>4</sup> abnormalities in hoof formation result from vitamin A deficiency.

<sup>1</sup> ZIMMERMAN, H. M., Lesions of the nervous system in vitamin deficiency. I. Rats on a diet low in vitamin A, *J. Exptl. Med.*, **57**, 215–228, 1933.

<sup>2</sup> SUTTON, T. S., H. E. SETTERFIELD, and W. E. KRAUSS, Nerve degeneration associated with avitaminosis A in the white rat, *Ohio Agr. Expt. Sta. Bull.* 545, 1934.

<sup>3</sup> GUILBERT, H. R., and G. H. HART, Minimum vitamin A requirements with particular reference to cattle, *J. Nutrition*, **10**, 409–427, 1935.

<sup>4</sup> KLEMOLA, VALTO, Über keratoplastische Reaktion der Hufhornbildung durch einige physiologische Faktoren, *Biedermann's Zentr. Abt. B. Tierernähr.*, **5**, 657–675, 1933.

**143. Vitamin A and "Cottonseed-meal Poisoning."**—Practical feeders early discovered that rations containing cottonseed meal were poisonous to swine and, in a lesser degree, to cattle, especially calves. In 1915 the toxic substance, gossypol, was isolated from cottonseed, and, on the basis of experiments with various species, principally laboratory animals, it became rather generally accepted as the causative agent in the troubles with cattle and hogs. It was early recognized, however, that, in cattle, the trouble occurred primarily on rations containing poor roughage and that animals on good pasture remained healthy. With the development of the knowledge of the requirements for minerals and vitamins, experiments were undertaken which showed that the symptoms in cattle, *viz.*, convulsions, stiffness, and blindness, could be produced with rations free from cottonseed products. It was also found that the troubles did not occur where rations containing cottonseed products were supplemented with plentiful intakes of high-quality roughage. In 1930 Halverson and Sherwood<sup>1</sup> at the North Carolina Experiment Station, where much of the previous work on gossypol had been done, reported a long-time investigation from which it was concluded that the failures in cattle ascribed to cottonseed-meal injury were due primarily to a deficiency of vitamin A. The effectiveness of sources of the vitamin in preventing and curing the symptoms was soon confirmed by several other workers.

In the case of swine, however, which were early recognized to be much more susceptible to the trouble, vitamin A has not proven effective. In a very extensive study, Robison<sup>2</sup> showed that neither sources of vitamin A nor various minerals were useful in preventing the typical symptoms of cottonseed poisoning in growing pigs, while distinctly different symptoms were produced on rations deficient in the vitamin.

It is clear that the final chapter on cottonseed-meal injury has not yet been written. Without doubt many, perhaps all, of the cases in mature cattle ascribed to it have been due to actual nutritional deficiencies, primarily vitamin A, but several studies suggest that the vitamin may not be the sole factor involved. It

<sup>1</sup> HALVERSON, J. O., and F. W. SHERWOOD, Investigations in the feeding of cottonseed meal to cattle, *N. C. Agr. Expt. Sta. Tech. Bull.* 39, 1930.

<sup>2</sup> ROBISON, W. L., Cottonseed meal for pigs, *Ohio Agr. Expt. Sta. Bull.* 534, 1934.

still remains doubtful whether cottonseed products can be fed in unlimited amounts to calves even though the ration is optimum in other respects. In so far as pigs are concerned, a nutritional deficiency does not appear to be the explanation, and the gossypol toxicity theory still stands.

The fact that the gossypol content of cottonseed varies according to soil and climatic factors and that it is destroyed in varying degrees according to the process used in expressing the oil is recognized to be responsible for some of the contradictory results obtained regarding the injury and to make practical recommendations difficult. Gossypol is clearly toxic to pigs. This may be true for cattle also but not at the low levels ordinarily present in the commercial meals. There may be physiological interrelations between gossypol and vitamin A and perhaps other nutritive factors. In hogs and rats, which are highly susceptible to the toxic agent, there is evidence of a lessened toxicity by certain dietary modifications. Treatment of cottonseed meal with iron salts certainly lessens its toxicity for hogs. A recent report by Robison<sup>1</sup> refers to the evidence for this conclusion and presents supporting data that autoclaving cottonseed meal destroys its toxicity for hogs. He has extended previous studies in showing beneficial effects from treatment of the meal with iron salts.

**144. The Chemical Nature of Vitamin A.**—Chemical isolation and identification early became a goal of vitamin research because of the recognized importance of working with pure substances of known composition, rather than with concentrates containing many other chemical compounds besides the vitamin under study. In view of the enormous difficulties involved in isolating substances which are complex in nature, present in only minute amounts, and rather unstable, it is not surprising that our knowledge of the chemical nature of the vitamins has lagged far behind their discovery. Remarkable progress has been made during the last decade with the result that today we have fairly complete information regarding several of these essentials, including vitamin A.

Vitamin A is a nearly colorless substance having the formula,  $C_{20}H_{30}O$ . Its structural formula has been provisionally worked

<sup>1</sup> ROBISON, W. L., Making cottonseed meal safe and effective when fed as the only protein concentrate to pigs in dry lot, *Proc. Am. Soc. Animal Production*, 1936, pp. 87-91.

out. The vitamin does not occur as such in plant materials, but rather as its precursors, certain carotenoid pigments which are converted into the vitamin in the animal body. The development of this knowledge represents a highly interesting chapter of vitamin research which illustrates the course of such studies.

In 1914 McCollum and Davis found that the vitamin was contained in the unsaponifiable fraction of milk fat and later studies confirmed its identity as an unsaponifiable constituent extractable by lipid solvents. In 1919 Steenbock called attention to the fact that among vegetable foods vitamin A potency was associated in a rather remarkable way with yellow color, and, shortly thereafter, he and his associates published many data demonstrating this association. They went so far as to suggest that carotene was the source of the vitamin. This view was not accepted by other workers, and Steenbock came to recognize that the vitamin was not carotene itself because certain potent sources of the vitamin were colorless. It was 10 years before the riddle was solved.

In the meantime studies in various laboratories gradually built up the body of information which provided the answer. Drummond and his associates used an old color reaction for cod-liver oil as a basis for developing a test which provided a method for the detection and estimation of vitamin A. The test involved the production of a brilliant blue color by arsenic trichloride. Later the test was modified by Carr and Price by the substitution of antimony trichloride for the arsenic compound and the test is known today as the *Carr-Price reaction*. The spectrograph (Sec. 20) also came into use for studying the nature of the vitamin, and its content in fish-liver oils was found to be correlated with a selective absorption with a maximum at 3280 Å. Both the color reaction and the absorption spectrum proved very useful in the later studies which resulted in the identification of the vitamin and in demonstrating its relationship to carotene.

Renewed beliefs in this relationship caused von Euler and associates<sup>1</sup> in Stockholm to conduct carotene-feeding experiments. They obtained a definite growth response when the pigment was added to a vitamin A deficient diet, and their results were quickly confirmed by several others. In 1930

<sup>1</sup> VON EULER, BETH, HANS VON EULER, and HARRY HELLSTRÖM, A-vitaminwirkungen der Lipochrome, *Biochem. Z.*, **203**, 370-384, 1928.



Moore<sup>1</sup> produced proof that the animal body transformed carotene into vitamin A. He fed rats on a diet which resulted in the symptoms of deficiency. Some of the animals were then killed, and their livers found devoid of A. The rest of the animals were fed carotene with a resulting disappearance of the deficiency symptoms, and, on autopsy, their livers were found rich in the vitamin. Moore summarized the information as follows:

Carotene	Vitamin A
Synthesized in plant	Stored in animal
Orange red in color	Almost colorless
No selective absorption in vicinity of 3280 Å.	Marked absorption at 3280 Å.
Greenish blue color with antimony trichloride, with maximum at 5900 Å.	Vivid blue color with antimony trichloride, showing maxima at 5270 and 6060 Å.

Meanwhile halibut-liver oil had been found an especially concentrated source of the vitamin and fractionation methods were applied to it to isolate the active substance. These methods brought success by 1932 in the isolation, by Karrer and his associates in Switzerland and by Drummond and his coworkers in England, of a very active fraction which was identified as an unsaturated alcohol having the formula,  $C_{20}H_{30}O$ . Karrer proposed the structural formula given on page 183. This alcohol, which was obtained in the form of a nearly colorless viscous liquid, is today accepted as the vitamin itself. Since it has not yet been crystallized, however, the possibility exists that impurities are still present.

There followed the brilliant researches by Karrer, by Kuhn in Germany, and by others which established the chemical structure of the highly complex hydrocarbon, carotene, which was previously known to have the empirical formula,  $C_{40}H_{56}$ . Several isomeric forms of carotene were isolated and their structural formulas worked out. All were reddish-yellow crystalline compounds which differed, however, as regards optical activity and the wave length at which maximum color absorption occurred. It is now recognized that there are four different carotenes which have vitamin A activity, viz.,  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and hydroxy- $\beta$ -carotene.

<sup>1</sup> MOORE, THOMAS, Vitamin A and carotene. I. The association of vitamin A activity with carotene in the carrot root, *Biochem. J.*, **23**, 803-811, 1929.





The last is also called kryptoxanthine. On hydrolysis  $\beta$ -carotene yields two molecules of vitamin A, but the other forms yield only one. Thus the  $\beta$ - form has twice the potency of the others. The preceding structural formulas given on page 183 indicate the relationship between  $\alpha$ -carotene,  $\beta$ -carotene, and the vitamin.

Such are the facts which appear established at this writing. Further research may modify them. Particularly, other precursors of vitamin A may be found. It is clear, however, that remarkable progress has been made since it became evident that vitamin potency was correlated with carotene content in plant products, but that certain rich animal sources were colorless. It is now evident that the potency of a given food may be due to the vitamin itself or to carotene or to both and that the potency varies according to the form of carotene present.

**145. Stability of Carotene and Vitamin A.**—Differing from the nutrients previously considered, the vitamins as a class are susceptible to destruction in varying degrees by certain physical and chemical agents which may become operative in the course of some of the processes to which feeds are subjected. The same is true during storage under certain conditions. The different vitamins vary greatly in their susceptibility to the action of these agents. Both carotene and vitamin A are destroyed by oxidation, and this is the most common cause of any depreciation which may occur in the potency of sources of them. The process is accelerated at high temperatures, but heat without oxygen has a minor effect. Butter exposed in thin layers in air at 50°C. loses all its vitamin A potency in six hours, but, in the absence of air, there is little destruction at 120°C. over the same period. Cod-liver oil in a tightly corked bottle has shown activity after 31 years, but it may lose all of its potency in a few weeks when incorporated in a feed mixture stored under the usual conditions.

This latter fact has an important practical bearing. Neither proprietary nor home-mixed feeds can be relied upon to contain the vitamin A potency originally present in the cod-liver oil or similar products included as an ingredient. The distribution of the oil over the other constituents provides a large surface for oxidative action, and this process is accelerated by the presence of prooxidants (Sec. 56) in any rancid fats present in the feed. Such fats in the oil itself are of course particularly operative.

Certain metals also, such as iron, catalyze the destructive action. Carotene appears to be less rapidly destroyed than the vitamin itself, but, when dissolved in oil and added to a feed stored at room temperature, losses as high as 68 per cent have been reported after 16 weeks. With both carotene and the vitamin, the nature of the associated substances has a marked influence on the rate of destruction. In all cases the action is retarded by storage at low temperature. Large losses in vitamin value as a result of oxidation take place in the curing of roughages and in certain other feed-preservation processes. The presence of prooxidants and antioxidants in the plant tissues plays a role in the extensiveness of the destruction. The cooking processes commonly used in human food preparation do not cause much destruction of the vitamin potency.

Hydrogenation of fats lessens their vitamin A value, and thus the commercial products prepared for culinary purposes commonly contain none of the vitamin, though it may have been present in the original material. Saponification does not destroy the vitamin if oxidation is avoided.

✓**146. Metabolism of Carotene and Vitamin A.**—Carotene is a product of plant synthesis which takes place in the lower forms including bacteria, as well as in the higher ones, but the conversion of the pigment into the vitamin is a function of the animal body only. The extent of the conversion varies with the species. It apparently takes place almost completely in rats, swine, sheep, and goats but only partially in cattle and chickens. It has been reported that the conversion does not take place at all in the cat, which suggests that the carnivorous animal may not be equipped to deal with a plant pigment. The seat of the conversion is considered to be the liver, but the earlier reports that this organ contains a carotenase as the responsible agent have received no support from more recent studies.

Both vitamin A and carotene can be stored in the animal body, and, in those species in which the conversion is only partial, a large storage of carotene occurs on diets rich in the pigment. The extensive and thorough studies of Guilbert and Hart<sup>1</sup> with cattle have contributed a large amount of information along these

<sup>1</sup> GUILBERT, H. R. and G. H. HART, Storage of vitamin A in cattle, *J. Nutrition*, **8**, 25-44, 1934; Minimum vitamin A requirements with particular reference to cattle, *ibid.*, **10**, 409-427, 1935.

lines. The total storage of carotene and vitamin A in the liver and depot fat of cows which had access to a carotene-rich ration throughout life was estimated to be 0.6 to 0.7 g. for the younger animals and up to 3.6 g. in aged cows. From 67 to 93 per cent of the storage was in the liver. In this organ most of the storage was in the form of the vitamin itself, while in the fat depots carotene predominated. A cow whose reserves were depleted was fed approximately 15 g. of carotene in freshly cut alfalfa during a period of 13 days and stored about 400 mg. It is evident that the rate of storage can greatly exceed the rate of depletion on a vitamin-deficient diet. Guilbert and Hart found that over 200 days elapsed before the livers of animals which had previously received feeds rich in carotene were completely depleted of the vitamin and its precursor. In studies with rats, Davies and Moore<sup>1</sup> have shown that the adult is able to store, with massive doses, enough vitamin A in its liver to supply its theoretical requirement for a century but that these superfluous stores are eliminated at a very rapid rate until a state of stable storage is reached.

The storage of vitamin A or its precursor or both appears to be a function of all species. It has a large practical importance in nutrition because it provides a means whereby reserves can serve during periods of dietary scarcity, and it eliminates the necessity of a constant daily supply of the vitamin or its precursor in the diet. A quantity sufficient to protect a rat for several months can be given in a single dose. Animals on good pasture can store extensive reserves to help meet their needs during the winter feeding period when their rations may be deficient. This large capacity to store the vitamin must be taken account of in studies of requirements in order to make sure that intakes that appear adequate for a given function are not being supplemented by reserves stored up prior to the period of observation.

✓ Among cattle there are marked breed differences as regards ability to convert carotene into the vitamin. This is reflected in the difference in color of the depot fat and milk fat between Holsteins and Jerseys. The bearing of this on the vitamin potency of the milk produced is discussed later (Sec. 329).

<sup>1</sup> DAVIES, ALAN W., and THOMAS MOORE, Vitamin A and carotene. XII. The elimination of vitamin A from the livers of rats previously given massive doses of vitamin A concentrate, *Biochem. J.*, **29**, 147-150, 1935.

**147. Quantitative Determination of Vitamin A Value.**—The improvement and standardization of methods for the quantitative estimation of the vitamin A value of foods constitute a very active field of research at the present time. Biological, chemical, and physical methods are being employed. Since the procedures are constantly being modified, the description of the methods in use will be limited to a statement of the principles involved.

The biological method measures the total potency whether due to the vitamin itself or to its precursor or to both, while certain others determine only one of these substances. The food to be tested is fed at several levels to different groups of young rats, as a supplement to a vitamin A free diet which has caused growth to cease. The growth response is then compared with that produced in a similar group of rats receiving as a supplement a standard source of the vitamin, such as  $\beta$ -carotene or a "reference cod-liver oil." In this way the amount of the feed under test which gives the same response as the standard supplement is determined and the potency is expressed in *units* per gram.

Various units have been employed for expressing vitamin A potency. This has resulted in some confusion and necessitates an understanding of the basis of each. The *Sherman-Munsell unit*, the first one in general use, is based directly upon the growth response with rats. One unit represents the daily intake which will cause an average gain in weight of 3 g. per week over an eight-week test. The *International unit*, set up in 1931, was the potency of 1 microgram of a sample of carotene, obtained by pooling samples prepared by workers in various countries. These samples were prepared before the differences in value of the various forms of carotene were appreciated. Later work has shown that 1 microgram of the pooled sample is equal to only 0.6 microgram of pure  $\beta$ -carotene which is now taken as the International standard. The Sherman-Munsell unit is equivalent to approximately 1.4 International units. The *A.D.M.A. unit* is equivalent to the Sherman-Munsell unit, and the same is true for the *U.S.P. X unit* formerly used by the U. S. Department of Agriculture. In 1936 the Department adopted the new *U.S.P. unit* which is the same as the International unit.

The biological method provides a direct measure of the actual utilizable vitamin potency. The method, in the standardized way that it is carried out with the rat, is hardly applicable for

employment with large animals. The values obtained with the rat are certainly useful as indicators of the relative value of various feeds for farm animals, even though the degree of utilization may vary in different species. The biological assay must be used as the criterion of the reliability of the chemical and physical methods of determining carotene and the vitamin itself. The latter methods provide a means for obtaining in a few hours information for which several weeks are required with the biological procedure. They have proved of inestimable value in speeding up the development of the knowledge of vitamin A.

The vitamin A present as such can be determined by a method based upon the Carr-Price reaction previously mentioned. Having extracted the vitamin, the intensity of the blue color obtained with antimony trichloride is compared with that produced by a standard of known potency. Vitamin A is also determined by measuring the coefficient of absorption at wave length 328  $m\mu$ . In this procedure a quartz spectrograph known as the Hilger vitameter is commonly used, and the method is primarily adapted to liver oils and their concentrates. Both of the above methods give fair agreement with a biological assay provided interfering substances are absent and provided certain precautions are observed.

The carotene present in feeds can be determined upon the unsaponifiable fraction of their extracts by colorimetric comparison with dye solutions standardized against  $\beta$ -carotene. This method has proven especially useful for the assay of green forage, because the vitamin potency of these feeds is due principally to  $\beta$ -carotene. Carotene can also be determined spectrographically. For the details of these methods the student should consult the current publications of the workers in this field.

✓ **148. Vitamin A Value of Feeds.**—While the chemical distinction between the vitamin itself and its precursors has proved highly important in working out their properties and physiological behavior, it is unnecessary to differentiate between them in considering the value of feeds as sources, because carotene becomes vitamin A in the body and thus both function alike in so far as the needs of the body for the vitamin are concerned. Thus the term vitamin A value is used in evaluating feeds even though a part of all of the potency may be in the form of the precursor.

Some swordfish-liver oils contain as many as 200,000 units of vitamin A per gram. Halibut-liver oil may run as high as 70,000

units. Thus both are many times more potent than cod-liver oils. Products from the same species, however, may be highly variable in potency, and thus, in their manufacture for use as vitamin A supplements, they are subjected to a biological assay in order that the user may be assured of a certain minimum potency. Among the common foods of animal origin, milk fat, egg yolk, and liver are rated as rich sources, but this is not the case if the animal from which they come has been receiving an A deficient diet for an extended period. Since the vitamin is present in the fat, skim milk contains very little.

In the nutrition of farm animals, we are primarily interested in the potency of plant products. Though the yellow color is masked by chlorophyll, all green parts of growing plants are rich in carotene and thus have a high vitamin A value. Good pasture always provides a liberal supply, and the kind of pasture plant, whether grass or legume, appears to be of minor importance. At maturity, however, leaves contain much more than stems, and thus legume hay is much richer in vitamin content than timothy or other grasses. With all hays and other forage, the vitamin value decreases after the bloom stage, and much of the carotene is destroyed by oxidation in the process of field curing. This latter loss has been studied especially by Russell.<sup>1</sup> There may be a loss of more than 80 per cent of the carotene of alfalfa during the first 24 hr. of the curing process. It occurs chiefly during the hours of daylight, owing to photochemical activation of the destructive process. Hays which are cut in the bloom stage or earlier and cured without exposure to rain or to too much sun retain a considerable proportion of their carotene content, while those which are cut in the seed stage and exposed to rain and to the sun for extended periods lose it almost entirely. Under similar conditions of curing, alfalfa and other legume hays are much richer than grass hays because of their leafy nature, but a poor grade of alfalfa may have less than a good grade of timothy. This is illustrated by the data given in Table XIII. The grade listed is based on color, and, in the case of alfalfa, on leafiness also. The data also show that fresh green alfalfa is much richer than the best cured product.

In the artificial curing of hay with a "hay drier," there is only a slight loss of carotene because of the rapidity of the process.

<sup>1</sup> RUSSELL, WALTER C., The effect of the curing process upon the vitamin A and D content of alfalfa, *J. Biol. Chem.*, 85, 289-297, 1929.

Russell and coworkers found the machine-dried product to have two to ten times the value of field-cured alfalfa. Severe heating of hay in the mow or stack reduces the vitamin content, and there is a gradual loss in storage so that old hay is poorer than new.

TABLE XIII.—CAROTENE CONTENT OF CERTAIN FARM FEEDS<sup>1</sup>

Feed	U. S. grade No.	Deter- mina- tions No.	Water, per cent	Carotene per gram of dry matter, mg.		
				High	Low	Average
Fresh green alfalfa.....	..	5	79.6	0.412	0.267	0.326
Alfalfa hay.....	1	6	8.6	0.117	0.034	0.045
Alfalfa hay.....	2	2	8.6	0.016	0.014	0.015
Alfalfa hay.....	3	2	8.6	0.012	0.001	0.007
Timothy hay.....	1	3	11.6	0.024	0.009	0.019
Timothy hay.....	2	1	11.6	.....	.....	0.008
Timothy hay.....	3	2	11.6	0.011	0.002	0.006
Fresh green Kentucky blue- grass.....	..	2	68.4	0.620	0.424	0.522
Fresh green corn plant; cut for ensiling.....	..	5	78.1	0.115	0.070	0.092
Corn fodder, old and dry....	..	2	9.0	0.006	0.002	0.004
Corn ripe grain, yellow dent, and flint.....	..	6	11.3	0.010	0.003	0.006
Carrots: yellow, garden.....	..	4	88.3	1.128	0.709	0.949

<sup>1</sup> MEIGS, E. B., Vitamin A value of plant feeds fully accounted for by their carotene content, *Yearbook of Agriculture, U. S. Dept. Agr.*, pp. 324-326, 1935.

Owing to their higher initial content machine-dried hays are subject to larger percentage losses than field cured. Losses as high as 60 per cent have been reported from July to November, but they were found much smaller during the colder months. Temperature is the major factor causing variations in loss during storage.

The data in Table XIII are useful to illustrate the differences that can occur between fresh and cured material and between hays of different kinds and grades. The large differences between the maximum and minimum values for the same product, however, show that the average values must not be taken too literally. This is generally true for the data in the literature for the vitamin A potency or carotene content of cured roughage.



No two samples are exactly alike because of the many factors operative in the destructive process. While the same hay contains much more potency when artificially dried than when field cured, a machine-dried product after a long period of storage may contain less than a freshly harvested, sun-dried product which has been cured under favorable conditions. In using published averages as a basis for feeding practice, the different factors which cause them to vary must be kept in mind.

As is indicated in Table XIII, though dry corn fodder is very low in carotene, the fresh plant as cured for ensiling is rich. The content in corn silage varies according to the stage of maturity and also according to the nature of the process which takes place in the silo, but it may be as rich as well-cured hay. Current research suggests that silage made by the new processes involving the addition of acid or molasses at the time of ensiling is richer in vitamin A potency than silage made in the regular way and that the acid product is superior to that made by molasses addition. Much further study with the various silage crops is required to establish the general applicability of these initial findings.

The degree of greenness in leafy material is a good index of its vitamin A potency. For example, Kramer and associates<sup>1</sup> found the outer green leaves of head lettuce thirty times as rich as the inner blanched leaves. Color is also a good measure of the potency of hays but, in storage, carotene is destroyed faster than the green color.

Aside from yellow corn and its by-products practically all of the concentrates used in feeding animals are devoid of the vitamin or nearly so. The potency of yellow corn which is due to the pigment, kryptoxanthine, is only about one-eighth that of good roughage. Roots and tubers as a class supply practically no vitamin A, but carrots are a very rich source and so are sweet potatoes, as might be expected from their yellow color. Pumpkins and squash also supply considerable amounts. The green leafy vegetables used in human nutrition are rich.

Tankage, meat scraps, and similar animal by-products have little if any vitamin A potency. Certain fish meals are fair sources, but variation in the raw material, and in the methods of

<sup>1</sup> KRAMER, MARTHA M., GLADYS BOEHM, and RUTH E. WILLIAMS, Vitamin A content of the green and white leaves of market head lettuce, *J. Home Econ.*, 21, 679-680, 1929.

processing which may entirely destroy any potency originally present, make generalizations here of no value.

#### VITAMIN D

Only a few years after vitamin A was discovered, it became clear, through the work of Mellanby in England with dogs, that a dietary deficiency was concerned in rickets. The proof that it was due to a distinct vitamin was furnished by McCollum and associates<sup>1</sup> in 1922. This proof was obtained by oxidizing cod-liver oil until vitamin A was destroyed, as shown by the inability of the oil to cure xerophthalmia, and then by demonstrating that the oxidized oil was still effective in curing rickets.

✓ **149. Physiological Effects of Vitamin D.**—In view of the circumstances of the discovery of vitamin D as well as the fact that cod-liver oil had long been known to be effective in the cure of rickets, the term, *antirachitic factor*, naturally arose. It is evident from previous discussions (Sec. 109) that, however the term is defined, rickets is a disturbance of calcium and phosphorus metabolism and that the mineral relations in the diet as well as the vitamin are involved. There can be no calcification without calcium and phosphorus. On the other hand, the vitamin has a nutritional significance in addition to its relation to this disturbance of bone growth.

✓ Undoubtedly vitamin D is always required for the normal calcification of the growing bone, but the amount needed varies with the mineral relations in the diet and also with the species. More is required when the amount of either element or the ratio between them is suboptimum. But no amount will compensate for severe deficiencies of either mineral. Theiler,<sup>2</sup> for example, has shown that rickets develops in calves on a low-phosphorus ration despite a very large supply of D in the form of radiant energy (Sec. 151). The species differences are illustrated by the fact that with adequate intakes of calcium and phosphorus a ration which contains only enough vitamin D to produce normal bone in the rat will very quickly cause the development of rickets

<sup>1</sup> MCCOLLUM, E. V., NINA SIMMONDS, J. E. BECKER, and P. G. SHIPLEY, Studies on experimental rickets. XXI. An experimental demonstration of the existence of a vitamin which promotes calcium deposition, *J. Biol. Chem.*, **53**, 293-321, 1922.

<sup>2</sup> THEILER, ARNOLD, The oosteodystrophic diseases of domesticated animals. I. The structure of the bone; atrophy; osteoporosis; osteomyelitis, *Vet. J.*, **90**, 143-158, 1934.

in chicks. Surprisingly, the human baby is more like the bird than the small mammal in this respect. Turkeys and pheasants have higher requirements than chicks. The effect of a lack of vitamin D and calcium on bone structure in young cattle is strikingly illustrated in Fig. 16.

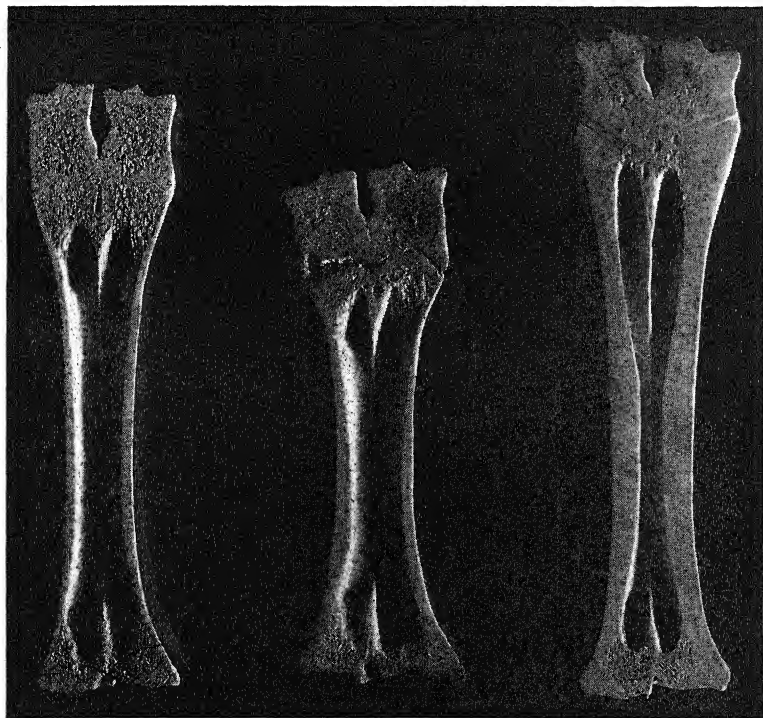


FIG. 16.—Effect of a deficiency of vitamin D and calcium on bone development in young cattle.

The bone on the right is from a normal animal; the other two from affected animals. Note the differences in thickness of walls and density. (From T. W. Gullickson, L. S. Palmer and W. L. Boyd. *A rickets-like disease in young cattle*. Minn. Agr. Expt. Sta. Tech. Bull. 105, 1935.)

While the necessity of vitamin D for normal calcification during growth has been demonstrated for many different species, it has not been found universally effective in preventing losses from the bones in the adult. Templin and Steenbock<sup>1</sup> have furnished evidence that the antirachitic factor markedly decreases the

<sup>1</sup>TEMPLIN, VERA M., and H. STEENBOCK, Vitamin D and the conservation of calcium in the adult. II. The effect of vitamin D on calcium conservation in adult rats maintained on low calcium diets, *J. Biol. Chem.* 100, 209–216, 1933.

losses of ash from the bones that otherwise occur in the adult rat on a diet very low in calcium. In lactating cows, however, in many experiments, no form of vitamin D has been at all effective in decreasing the losses of calcium and phosphorus which take place at the height of lactation on a ration rich in the elements (Sec. 332). The ineffectiveness here is not due to a failure of absorption of the vitamin. In contrast, there is experimental evidence that the vitamin is effective in the lactating goat under the same conditions where it is of no value in cows. There is evidence for a positive effect also in the case of nursing mothers, although not to the extent of causing calcium and phosphorus equilibrium in all cases. It is understandable that the vitamin may be more effective in the formation of new bone during growth than in regulating the deposition and mobilization of calcium and phosphorus in the trabeculae.

The evidence is conflicting as to the role of the antirachitic factor in the healing of fractures. Large intakes have been reported helpful in promoting the calcification of the tubercles in the treatment of tuberculosis. The vitamin has also been found effective under certain conditions in retarding the development and spread of dental caries, but several other factors are clearly involved.

A lack of the antirachitic factor decreases egg production and hatchability, and the few eggs laid have thin shells and are easily broken. The vitamin content of the eggs produced is influenced by the amount present in the diet of the hen (Sec. 294). There is no evidence that the factor plays any role in milk secretion, but the amount present in milk can be influenced by the nature of the diet (Sec. 332). Apparently the vitamin is not readily secreted into milk, for massive doses in the feed are required to influence its concentration in this secretion. Ordinary levels of cod-liver oil are ineffective for this purpose, and the same is true for irradiation of the animal.

It is evident that the body has some ability to store the vitamin, though to a much lesser extent than is the case for vitamin A. Vitamin D is found in the blood, liver, lungs, kidneys, and elsewhere. Only a limited amount is stored in the fetus. When the human mother receives liberal intakes of the vitamin during pregnancy, the child is somewhat less susceptible to rickets, but protection cannot be provided in this way for any extended period.

**150. Mode of Action of Vitamin D.**—There is no specific information at the present time as to just how and where vitamin D functions in aiding calcium and phosphorus metabolism. It was early noted that in rickets the pH of the intestinal contents and of the feces was more alkaline than normal and that this condition was corrected by the ingestion of vitamin D. This observation, in view of the recognized effect of an acid reaction in promoting calcium and phosphorus absorption, led to the view that the vitamin functioned by increasing absorption. Later work has failed to bear out this explanation and certain facts appear to make it untenable. Clearly, the vitamin increases net absorption in the sense that more of the minerals remain in the body and that their levels in the blood may be increased, but this is a statement of facts rather than an explanation of the mechanism. The vitamin itself is absorbed and circulates in the blood stream, and thus its site of action may be anywhere in the body. The similarity of some of the effects of the antirachitic factor and parathyroid hormone is responsible for the theory that the vitamin acts through the parathyroid glands. But the effects of the two agents on the chemistry and histology of bone are very different, and it seems improbable that there is any direct relationship between them. It has also been suggested that the vitamin acts by altering the relations of the different forms of calcium in the blood (Sec. 106).

Much of the ingested vitamin appears in the feces, but there is no way of knowing what part of this is actually unabsorbed. There is little or no excretion in the urine (cow).

**151. Vitamin D and Radiant Energy.**—While the value of sunlight in the treatment of rickets had been known for many years prior to the discovery of vitamin D, it was not until X-ray methods of diagnosis became available that positive proof was obtained of a specific effect on bone calcification. Using these methods Huldschinsky<sup>1</sup> demonstrated in 1919 that ultraviolet light caused the deposition of calcium salts in the bones of rachitic children and thereby cured the disease. Later work showed that sunlight was also effective.

When it became evident that both ultraviolet light and a factor present in cod-liver oil produced an identical effect in the healing

<sup>1</sup> HULDSCHINSKY, K., *Heilung von Rachitis durch künstliche Höhensonne*, *Deut. med. Wochschr.*, **45**, 712-713, 1919.

of rickets, the question naturally arose as to why two such apparently unrelated factors could produce the same specific results. The answer was not long in coming. Following the studies of Goldblatt and Soames showing that the livers of irradiated rats possessed antirachitic properties, Hess of Columbia University and Steenbock of Wisconsin, independently and almost simultaneously, announced in 1924 that food materials which were ineffective in preventing rickets could be made antirachitic by exposing them to ultraviolet light. The original announcements were published in detail by Hess and Weinstock,<sup>1</sup> by Steenbock and Black<sup>2</sup> and by Steenbock and Nelson.<sup>3</sup> Several other papers by Steenbock, Hess, and their associates quickly followed showing that a great variety of edible materials could be activated, that the same short-wave lengths were here concerned as were effective in irradiating the body and that the active substance was in the unsaponifiable fraction. These observations provided the working hypothesis that radiant energy cured rickets because it activated some precursor in the body to provide the active agent, that by similar action it produced in certain foods a similar agent which became effective upon ingestion, and that certain substances such as cod-liver oil naturally possessed this agent. Leads were thus provided for an attack on the problem of the chemical nature of the antirachitic factor.

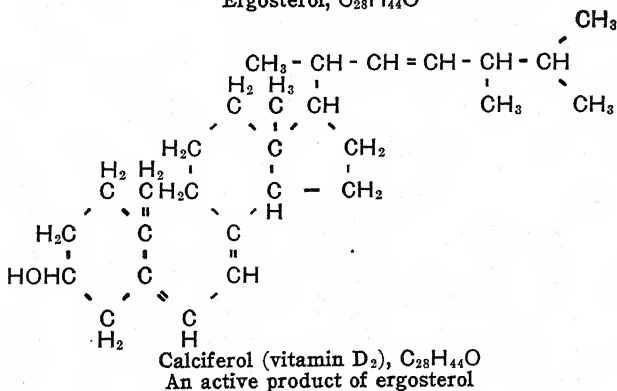
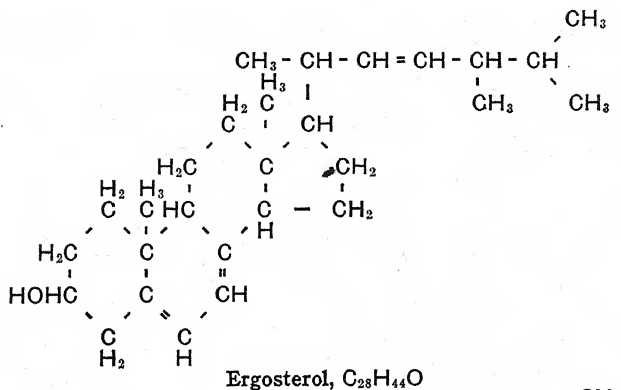
**152. Chemistry of Vitamin D.**—The knowledge that the active substance in materials made antirachitic by irradiation was in the nonsaponifiable fraction led to experiments with various sterols which resulted in the initial conclusion that cholesterol was the specific one activated. Later studies showing that irradiated ergosterol was much more active were responsible for the view that the activity of cholesterol was due to ergosterol as a contaminant. Several different kinds of evidence from various laboratories led to the conviction by 1928 that ergosterol was the

<sup>1</sup> HESS, A. F. and MILDRED WEINSTOCK, Antirachitic properties imparted to inert fluids and to green vegetables by ultra-violet irradiation, *J. Biol. Chem.*, **62**, 301-313, 1924.

<sup>2</sup> STEENBOCK, HARRY, and ARCHIE BLACK, Fat-soluble vitamins. XVII. The induction of growth-promoting and calcifying properties in a ration by exposure to ultra-violet light, *J. Biol. Chem.*, **61**, 405-422, 1924.

<sup>3</sup> STEENBOCK, HARRY, and M. T. NELSON, Fat-soluble vitamins. XIX. The induction of calcifying properties in a rickets-producing ration by radiant energy, *J. Biol. Chem.*, **62**, 209-216, 1924.

precursor which became vitamin D through a molecular rearrangement brought about by the action of radiant energy. Attention was next directed to the very difficult task of isolating the active substance present in the resinous gum which resulted from the most effective irradiation of the crystalline ergosterol. Success was achieved by 1932. A group of English workers isolated from the mixture of sterols, which resulted from irradiation of ergosterol, a highly active crystalline substance which they named *calciferol*, and an essentially identical substance was simul-



taneously obtained by Windhaus and associates in Germany. Calciferol melts at 114 to 117°C. and is isomeric with ergosterol, which melts at 166°C. As is indicated by the accompanying formulas ergosterol has three double bonds while calciferol has four. Thus irradiation results in breaking one of the carbon-to-



carbon linkages. There is not complete agreement as to where this break occurs, and thus the formula for calciferol is still tentative.

The isolation of calciferol led to the general belief that the question of the identity of vitamin D had been solved, although it was recognized to be only one of the irradiation products of ergosterol. In the meantime, however, other experimental evidence commenced to accumulate which now makes it clear that calciferol is not the sole form of vitamin D and that ergosterol is not the sole provitamin. It was shown by several workers that when dosages of cod-liver oil and irradiated ergosterol which were of equivalent effectiveness in rats (*i.e.*, dosages containing the same number of rat units) were fed to chicks, the oil was many times more effective. The chick was found to rank between the rat and the chick as regards its response to the two forms of the vitamin. Differences of the same nature among the three species were obtained when milks enriched in vitamin D in different ways (Sec. 333), *viz.*, by irradiation, by feeding irradiated food to the cow and by adding a concentrate of cod-liver oil directly to the milk, were compared. Butterfat was found much less effective for the chick than the rat equivalent of cod-liver oil. While it was recognized that these differences might have been due in part to the interplay of other dietary variables or to species variations in absorption and utilization, their magnitude clearly indicated that more than one form of the vitamin was concerned.

This conclusion as to the multiple nature of the vitamin received important support from other directions. It was found that irradiated cholesterol, the activity of which had become accepted as due to ergosterol contamination, was much more effective for chicks than the rat equivalent of irradiated ergosterol itself. Liver oils from different fish species were found to vary in chick potency per rat unit. It was found that 22 dihydroxy-ergosterol and 7-dehydrocholesterol could be activated by irradiation. The activation of cholesterol and ergosterol by chemical treatment was achieved.

These studies, which are being actively continued, make it clear that the question of the chemical nature of vitamin D is much more complicated than was thought a few years ago. The changing concepts of the last five years suggest that few positive statements are safe at the present time. There appears to be no



doubt that several forms of the vitamin occur and the possibility exists that there are many molecular configurations which exert an antirachitic effect. There is no evidence that any of the previously mentioned activated sterols is identical with the one or more forms of the vitamin which occur naturally in fish-liver oil, eggs, and other animal products. Within the past year, workers in two laboratories have reported the isolation from tuna-fish-liver oil of an active substance or substances which may be the same as one or more of the natural vitamins D. The name, vitamin D<sub>3</sub>, has been proposed for the product obtained from tuna-liver oil to distinguish it from calciferol which is referred to as vitamin D<sub>2</sub>. What sterol or sterols are activated when the body is subjected to radiant energy remains unknown. An extensive review of the experiments demonstrating the multiple nature of vitamin D is to be found in the publications by Bills.<sup>1</sup> In the second publication, he announces the irradiation of a new provitamin obtained from sitosterol. Bills states that at least eight forms of vitamin D have been artificially prepared and that there are at least two forms in fish oils which may or may not be the same as the artificial products. For the most recent developments, the current publications of workers in the field must be followed.

**153. Vitamin D Assay.**—The antirachitic value of a food can be measured either by chemical analysis of the bones or by X-ray methods or by the "line test." The latter, originally developed by McCollum and associates,<sup>2</sup> is the one at present recommended by the U. S. Pharmacopoeial Vitamin Advisory Board. The deposition of calcium salts in the metaphyses of the distal ends of the radii and ulnae of growing rats is measured by the extent of the staining of sections by silver nitrate. Standard rats are first made rachitic on a basal ration, and then the substance to be tested is added in graded amounts to different groups and the calcification compared with that obtained in a group receiving a reference cod-liver oil of known potency. For the details of the

<sup>1</sup> BILLS, CHARLES E., Physiology of the sterols, including vitamin D, *Physiol. Rev.*, **15**, 1-97, 1935; New forms and sources of vitamin D, *J. Am. Med. Assoc.*, **108**, 13-15, 1937.

<sup>2</sup> MCCOLLUM, E. V., N. SIMMONDS, P. G. SHIPLEY, and E. A. PARK, Studies on experimental rickets. XVI. A delicate biological test for calcium-depositing substances, *J. Biol. Chem.*, **51**, 41-49, 1922.

test, the student is referred to the most recent announcement of the advisory board.

Vitamin D potency is expressed in rat units per gram. The U.S.P. unit, which is also the International unit, is the activity of 1 mg. of the International standard solution, prepared under conditions defined by the League of Nations Health Organization. The older Steenbock unit is approximately equivalent to 3.3 U.S.P. units. Expressing potency in rat units is useful for standardization and control purposes, but it should be remembered that equivalence in rat units does not mean the same effectiveness for different sources in all species.

Because of the marked differences in the response of poultry to different sources of the same rat potency and in view of the wide use of vitamin D supplements in poultry rations, these supplements are frequently tested by the manufacturer with chicks and sold on the basis of their potency with this species.

✓ **154. Vitamin D in Foods.**—Of all the vitamins, the antirachitic has the most limited distribution in natural foods. Among animal products, eggs, especially the yolks, are a very good source, particularly where the diet of the hen is rich. Milk contains a variable amount in its fat fraction (5 to 40 U.S.P. units in cow's milk per quart), but neither cow's nor human milk contains enough to protect the baby against rickets. Other animal products are poor, as is to be expected from the fact that the storage of the vitamin in animal tissues is very limited. Certain fish meals, depending upon the nature of the raw material and its processing, contain fair amounts of the antirachitic factor.

✓ Seeds and their by-products are practically devoid of the vitamin. So are growing crops, including pasture grass. During the sun curing of roughages, however, vitamin D is formed under the action of radiant energy upon ergosterol or some other provitamin, and the principal source of the antirachitic factor in the rations of farm animals is thus provided. Legume hay which is cured in such a way as to preserve most of its leaves and green color contains considerable amounts. Timothy and other grass hay contain less. Stemmy hay, lacking in leaves and color, which has been exposed to a minimum of sunlight may contain none, whether legume or nonlegume. Artificially dried hay contains much less than that which has been properly sun cured. Corn silage contains a demonstrable amount of the vitamin,

primarily in the portions, such as the tassels, silk, and leaves, which are dried in the field. Green leafy vegetables contain little or none of the antirachitic factor, contrary to popular ideas. Neither do roots nor tubers. Recent studies by Kon and Henry<sup>1</sup> have shown that cacao-shell meal, a sun-dried by-product of chocolate manufacture, is a remarkably rich source. The meal contained 35 International units or more per gram, and its fat contained 300 units.

Cod- and certain other fish-liver oils, as well as certain fish-body oils, are rich sources of vitamin D and thus are used in both human and animal nutrition to supplement the common foods which are deficient. These oils are of variable potency. The U.S.P. specifications for cod-liver oil provide that it shall contain at least 85 units per gram. Many samples are much more potent than this, and the original potency is multiplied many times in the concentrate, consisting primarily of the unsaponifiable fractions, which are prepared for marketing in tablets, capsules and other forms for human consumption. A concentrate of fish-liver oils containing three million units per gram has recently been announced.

Vitamin D is much more stable than vitamin A. Heat has little effect and oxidative destruction occurs only very slowly. There is no marked loss from roughages on storage. Cod-liver oil mixed into feeds retains its antirachitic potency unimpaired for many months, in contrast to the marked destruction of vitamin A which occurs under the same conditions.

**155. Enrichment of Foods in Vitamin D.**—Steenbock patented his discovery that certain foods could be enriched in the antirachitic factor by irradiation with ultraviolet light, assigning the patent to the University of Wisconsin. The process has found wide application and has proved an outstanding contribution to the better nutrition of children. Activation is dependent upon the presence of a provitamin in the substance in question, and thus certain materials develop a high potency on irradiation, while others acquire little or none. The most potent product is obtained by irradiating ergosterol itself. This product is sold in various forms under the name "viosterol." Milk, which normally

<sup>1</sup> KON, STANISLAW K., and KATHLEEN M. HENRY, The effect of feeding cacao shell to cows on the vitamin D content of butter (milk), *Biochem. J.*, **29**, 2051-2056, 1935.

is not a rich source of the vitamin, can be irradiated to contain 135 U.S.P. units or more per quart, which is ample as a daily intake to protect a child against rickets. Yeast is rich in ergosterol and thus develops a high D potency on irradiation. Cereals, bread, and various other foods are also irradiated.

In the irradiation process, a quartz mercury-vapor lamp or a carbon arc provides the ultraviolet rays. The process requires a very careful control as regards wave lengths, time of exposure, and other conditions in order to obtain a product of maximum potency. The most effective wave lengths are the shorter ones of the ultraviolet range. Ergosterol absorbs strongly from 305 to 230  $m\mu$ . In the irradiation of ergosterol, various other sterols are formed as intermediate products which are inactive, and the active substance is itself destroyed by too long exposure. In addition to the process covered by the Steenbock patent, activation can be brought about by cathode rays. A vitamin D concentrate from ergosterol is commercially produced by this method, which is also controlled by patents.

Foods can also be enriched in the antirachitic factor by adding to them a concentrate prepared from one of the rich natural sources such as cod-liver oil. At least two different methods, controlled by patents, are used to obtain the unsaponifiable fraction which contains the vitamin, and various products produced in this way are marketed for human and animal feeding. Using cod-liver oil as the source, the concentrates are rich in vitamin A as well as D. They are added to milk, bread, and other foods.

Because cheaper and more practicable methods of supplying vitamin D are available, none of these irradiated products are commonly used in animal feeding with the exception of irradiated yeast which is used for the production of a milk of high antirachitic potency for human consumption (Sec. 333). "Reinforced" cod-liver oils, guaranteed to contain a minimum of 400 U.S.P. units of vitamin D and 3000 U.S.P. units of A, find considerable use for animals, especially in poultry feeds.

**156. Sunlight and Vitamin D Nutrition.**—The previous statements that most of the commonly used feeds contain little or no vitamin D suggest that there must be a widespread need for supplements of the vitamin in feeding farm animals. This would be true were it not for the sun, which by irradiation produces the factor from precursors present in the body. In sunlight, nature

has provided for the deficiencies in most of her food products. The skin and sebaceous secretions contain the provitamin, and thus the activated substance is produced on and in the skin from which it is absorbed. That this absorption can take place is clear from the fact that rickets can be successfully treated by rubbing cod-liver oil on the skin. Animals also ingest some of the activated material present in the skin secretions in the process of licking the body.

The effectiveness of the sunlight is dependent upon the lengths and intensity of the ultraviolet rays which reach the body. It is ineffective through ordinary window glass because the latter does not allow sufficiently short wave lengths to pass through. The radiations which reach the earth contain only a small part of the ultraviolet range which has an antirachitic effect. The shortest wave length which ever reaches the earth is  $290\text{ m}\mu$ , shorter ones being absorbed by the atmosphere. This shortest available wave length reaches the earth only in summer and only in the tropics. The greater the distance the rays have to travel, the longer is the minimum wave length which reaches the earth and the lesser the intensity of the effective radiations. Thus sunlight is more potent in the tropics than in the Temperate or Arctic zone, more potent in summer than in winter, more potent at noon than in the morning or evening and more potent at high altitudes. These variations are of large importance in vitamin D nutrition. Animals which are on pasture during the summer never suffer from the lack of the antirachitic factor even though their diet is practically devoid of it. In the wintertime the story is different. At best, the animals are outside only a part of the time, there are generally fewer sunny days, and the sunlight which actually reaches the animal is much less effective than in summer. Under most conditions of practice in the latitude of the northern United States, it is unsafe to rely on exposure to sunlight to provide the antirachitic factor during the winter months.

Fortunately, especially for city dwellers, it is not necessary for the body to be in the direct sunlight in order that activation may take place. It can occur in the shadow on sunny days. "Sky-shine" from the northern sky on bright days may be one-half to two-thirds as potent as direct sunlight. Rays reflected from snow and water are more potent than when direct. Clouds, smoke, and dust, however, greatly cut down the effectiveness of the light.

The dust and smoke of cities are responsible for the much greater incidence of rickets in city children than occurs in the country.

Ordinary clothing, particularly of men, allows little penetration by the effective ultraviolet rays. In animals, irradiation is much more effective on exposed skin than through a heavy coat of hair or wool.

**157. Overdosage with Vitamin D.**—Experiments with massive doses of irradiated ergosterol have shown that a condition of "hypervitaminosis" can be produced characterized by hypercalcemia, the widespread deposition of calcium salts in the arteries and various organs and tissues, other pathological changes, and even death. Fortunately the range between the body requirements and the harmful dose is rather wide. The lethal dose is around ten thousand times the normal requirement, and the harmful dose is at least several times this requirement. It has been clearly shown that the toxic factor is the vitamin itself. There need be no fear from intakes of the vitamin which represent the maximum ever needed for normal calcification, but, since harm can result from overdosage, it is important to bear in mind that excessive intakes may be injurious rather than beneficial. Harm can also result from overirradiation of the body.

#### VITAMIN E

As a result of the stimulus to experimentation with purified diets which followed the discovery of the first vitamin, it was frequently observed that on certain diets, which were satisfactory for growth and health, rats failed to reproduce. Studies of the cause of this failure resulted in the discovery by Evans of the University of California, Mattill of the University of Rochester, and Sure of the University of Arkansas, and their associates that there is a specific dietary factor essential for reproduction in the rat which is different from any of the other vitamins. Credit is due particularly to Evans and his group for the detailed histological and other studies showing specifically how a lack of the vitamin upsets the cycle of physiological events which combine to provide normal reproduction. They first designated the essential factor as "X" but it later became known as vitamin E. A comprehensive review of the experiments dealing with the dis-

covery of the vitamin and with its physiological effects, distribution in foods, and properties was published by Evans<sup>1</sup> in 1932.

**158. Physiological Effects of Vitamin E.**—It has been definitely proved that vitamin E is essential for normal reproduction in the rat, mouse, and in poultry, both male and female. When the factor is absent from the diet of the female rat, death and resorption of the fetuses result. In the male, degenerative changes in the testes are produced. The injury of the male causes permanent sterility, while in the female later pregnancies are successful if the vitamin is supplied. These pathological changes are described in some detail in Chap. XIV, in which the physiology of reproduction is outlined. While a lack of the vitamin results in reproductive failure, no amount of it has any effect in producing larger or more vigorous litters.

In the rat, if the deficiency of the vitamin is not so great as to cause fetal death, it may nevertheless result in paralysis in the suckling young toward the end of the nursing period. Several experiments have suggested that this is due to a lack of the vitamin in the milk secreted, which would mean that the vitamin is required for growth, at least during the suckling stage. No one has demonstrated any immediate need for the factor for rat growth after weaning. Particularly significant are the recently reported negative results by Olcott and Mattill.<sup>2</sup> Some observers report an impairment of vigor, muscle atrophy, and other signs of poorer health in adult animals reared on diets lacking in the vitamin, which suggest that it may have some beneficial effects other than in reproduction.

The rat has a large ability to store vitamin E in its tissues. This is shown by the fact that females born of mothers whose diets contained a liberal supply frequently have enough in their bodies at birth to carry them through a first pregnancy. Rats reared on natural foods rich in the factor and then placed on a deficient diet may produce three or four litters before exhausting their reserves. The vitamin E content of the body tissues can also be demonstrated by feeding these tissues to females which have failed as a result of a deficiency and noting the recovery that occurs. In the

<sup>1</sup> EVANS, HERBERT McLEAN, Vitamin E, *J. Am. Med. Assoc.*, **99**, 469-475, 1932.

<sup>2</sup> OLCOTT, H. S., and H. A. MATTILL, Vitamin E and early growth. *J. Biol. Chem. Proc.*, **114**, lxxvii, 1936.



past, a lack of appreciation of this large capacity for storage was responsible for several contradictory reports regarding this factor and its distribution in foods. Storage doubtless complicates a study of questions now at issue as to the specific need of the vitamin for any other function than reproduction. It has been shown that the stores are gradually used up in the regular metabolic processes of the animal whether or not a pregnancy is imposed.

**159. Chemistry of Vitamin E.**—Much progress has been made in the last few years in preparing very active concentrates of vitamin E and in determining its chemistry. These contributions have been made by Olcott, Mattill, Drummond, Evans, and others. In a publication which reviews earlier work, Olcott and Mattill<sup>1</sup> have described a concentrate from the nonsaponifiable fraction of wheat-germ oil which is potent in a single dose of 3 mg., and later work by Olcott showed the vitamin to be an alcohol. Evans and coworkers<sup>2</sup> have very recently reported the isolation of an alcohol having the properties of vitamin E and effective as a single dose of 3 mg., which they call  $\alpha$ -tocopherol. They propose the provisional formula,  $C_{29}H_{50}O_2$ .

Vitamin E is stable to dry heat at 250°C., to aeration at 97°C., to cooking, to light, and to some forms of mild oxidation, but it is readily destroyed in the presence of fats that go rancid. This effect is counteracted in wheat-germ oil by the presence of antioxidants. Ferric salts rapidly destroy the vitamin. Lard is a fat which readily promotes its inactivation. This rapid destruction which occurs under certain conditions was responsible for some early disputes regarding the existence and occurrence of the factor.

**160. Vitamin E in Foods.**—Vitamin E is widely distributed in the common foods. Green forage and other leafy materials are excellent sources. Alfalfa, spinach, lettuce, watercress, and the like are especially rich. The vitamin is abundant in cereal grains and other seeds especially in the germ. It is also present in various animal products. Wheat-germ oil is the most con-

<sup>1</sup> OLCOTT, H. S., and H. A. MATTILL, Vitamin E, I. Some chemical and physiological properties, *J. Biol. Chem.*, **104**, 423-435, 1934.

<sup>2</sup> EVANS, H. M., O. H. EMERSON, and G. A. EMERSON, The isolation from wheat germ oil of an alcohol,  $\alpha$ -tocopherol, having the properties of vitamin E, *J. Biol. Chem.*, **113**, 319-332, 1936.



centrated source, but cottonseed oil and corn oil are also rich. Milk is relatively poor in the vitamin, but egg yolk is rich, if the hen's diet contains the vitamin liberally. Various feeds used in dairy rations have been studied by Hathaway and Davis.<sup>1</sup> Fertility was restored in rats when their ration contained only 20 to 25 per cent of any of the following feeds: wheat bran, wheat shorts, linseed meal, hominy feed, white or yellow corn, kafir grain, alfalfa hay.

In testing a food for vitamin E, females are put on a vitamin E free ration at weaning and kept on it until 90 to 120 days of age, at which time they are mated to males of proven fertility. The animals which fail to produce young under these conditions, but nevertheless show the typical weight changes indicative of the start of fetal growth and its later cessation with resorption, are used to test the substance in question. The vitamin E content is determined by noting the ability of graded amounts of the test substance to cause the birth of a normal litter from females which previously have shown ability to conceive but have shown failure during gestation.

#### THE ANTINEURITIC FACTOR (VITAMIN B OR B<sub>1</sub>)

The early work of Eijkmann and others which established the fact that there is a specific dietary factor essential for the prevention of beriberi in man and polyneuritis in pigeons has been referred to. Further studies of this factor, which became known as water-soluble B and later simply as vitamin B, caused it to be recognized as essential for growth and for certain other physiological functions besides its antineuritic properties. As more critical investigations were made of its distribution in foods, chemical nature, and properties, the realization gradually developed, between 1925 and 1930, that vitamin B actually consisted of at least two factors differing as regards chemical nature and physiological effects. Thus what was formerly spoken of as vitamin B came to be called the *vitamin B complex*. The term, vitamin<sup>2</sup> B

<sup>1</sup> HATHAWAY, I. L., and H. P. DAVIS, The vitamin E content of certain dairy feeds, *Neb. Agr. Expt. Sta. Research Bull.* 73, 1934.

<sup>2</sup> In the report of the committee on vitamin nomenclature of the American Society of Biological Chemists and the American Institute of Nutrition, approved by these societies in April, 1937, it was recommended that the letter designation for this vitamin be dropped and that it be called *thiamin*, a term indicative of its chemical nature.

(or B<sub>1</sub>) was reserved for the antineuritic factor, and a new term, vitamin G or B<sub>2</sub>, came into use to denote a separate water-soluble factor which for a time was considered to be essential both for growth and for the prevention of certain skin lesions in different species.

This differentiation necessitated a repetition of much of the former work on distribution, nature, and properties, which had been carried out before it was recognized that more than one factor was involved. These studies soon made it apparent that there were still other water-soluble factors, in what continues to be referred to as the vitamin B complex or group. Especially during the last three years, new ones have been proposed with bewildering rapidity. Today, the literature contains the names of more than a dozen such factors and others are doubtless on the way. It seems improbable that all which have been reported actually exist or are actually different, but there is no basis for deciding at the present time what ones will be retained and what ones will be discarded when the story of the vitamins is complete. Most of the recently reported water-soluble factors have been proposed on the basis of work with one species only, generally the rat or chick, and there is no information at all regarding their need by farm animals. At the present time, only two of the factors of the B complex are sufficiently well characterized to permit any detailed discussion. One of these is clearly the antineuritic factor.

**161. Species Requiring Vitamin B (B<sub>1</sub>).—**Studies have definitely shown that the antineuritic vitamin is required by man, poultry, pigeons, rats, mice, and dogs. There is a lack of information regarding its need by larger animals because the necessary studies have not been made. Scheunert has recently reported that it is required by the lion to prevent "star-gazing disease," so named because a head retraction is involved.

In 1926 Bechdel and coworkers<sup>1</sup> reported that a ration which was so low in the vitamin B complex as to stop growth and to cause death in two to five weeks in rats was adequate to grow calves normally to maturity and to permit reproduction. They concluded that the calf must either have a very low requirement compared to the rat, or that the vitamin was produced by

<sup>1</sup> BECHDEL, S. I., C. H. ECKLES, and L. S. PALMER, The vitamin B requirement of the calf, *J. Dairy Sci.*, 9, 409-438, 1926.

synthetic action in the digestive tract (Sec. 41). Damon had previously shown that certain bacteria possessed this synthetic power. To study the question of vitamin synthesis during digestion in the cow, Bechdel and coworkers<sup>1</sup> made use of the rumen-fistula method originated by the French physiologist, Colin. They found that rats receiving a control ration supplemented with an extract of rumen contents from an animal receiving a ration deficient in the B complex made a much better growth than those receiving the vitamin-deficient control ration alone. They also isolated the predominantly occurring bacterium in the rumen contents and showed that the dead cells resulting from its growth on a vitamin-free medium were rich in the growth factor. Though these experiments were carried out before the multiple nature of the vitamin B complex was fully appreciated, it seems probable that at least one growth factor being dealt with was the anti-neuritic. To what extent this symbiotic action in the rumen of the cow occurs with other ruminants cannot be stated.

In connection with their studies of synthetic diets for Herbivora, Madsen and coworkers<sup>2</sup> observed that sheep required some alcohol-soluble factor of the B complex present in yeast. It is very possible that the rumen flora on a synthetic diet is different from that on a ration of natural feeds and that this requirement would not exist on the latter. Further work is highly desirable on the question of the need of ruminants for the specific factors of the vitamin B complex which are now recognized to be required by the omnivorous laboratory animals.

**162. The Physiological Effects of Vitamin B (B<sub>1</sub>).—**An early symptom of vitamin B deficiency is a loss of appetite (anorexia). This appears to be a result of a decreased secretion of the gastric and other digestive juices, and there is some evidence that the vitamin plays a regulatory role in connection with their secretion. The decreased food consumption which results from the loss of appetite causes in turn a marked retardation of growth. According to the work of Sure a lack of vitamin B has a direct effect on

<sup>1</sup> BECHDEL, S. I., HANNAH E. HONEYWELL, R. ADAMS DUTCHER, and M. H. KNUTSEN, Synthesis of vitamin B in the rumen of the cow, *J. Biol. Chem.*, **80**, 231-238, 1928.

<sup>2</sup> MADSEN, LOUIS L., C. M. MCCAY, and L. A. MAYNARD, Synthetic diets for Herbivora, with special reference to the toxicity of cod-liver oil, *Cornell Agr. Expt. Sta. Mem.* 178, 1935.

growth as well as that exerted through a loss of appetite. At any rate, a marked retardation of growth is a characteristic symptom. In the rat a severe deficiency may result in growth failure and death before other symptoms become apparent.

The nervous symptoms implied by the term antineuritic represent a later stage of the deficiency and are somewhat different in different species. Beriberi as it occurs in the Orient is seldom if ever an uncomplicated vitamin B deficiency. This is apparent from the nature of the diet and from the fact that cases may not respond to vitamin B ingestion alone, or they may respond very slowly, in contrast to the remarkable rapidity (within a few hours) which characterizes the cure of experimental polyneuritis in birds.

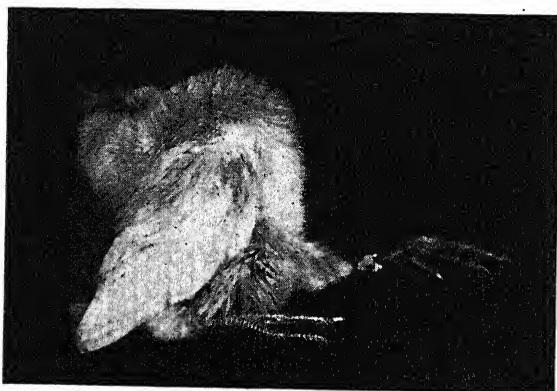


FIG. 17.—Polyneuritis. (Courtesy of L. C. Norris, Cornell University.)

In the latter species, there is a typical head retraction (Fig. 17) as well as convulsions and inability to stand. Muscle incoordination and paralysis occur in rats and dogs. There are conflicting reports as to the specific pathological changes responsible for the nervous symptoms. This is not surprising since so much work on this question was done before the multiple nature of the original vitamin B was appreciated. Contrary to earlier ideas, it now seems to be accepted that there is no marked degeneration of peripheral nerves but that the disturbance lies in the central nervous system. If this degeneration were severe and widespread, the rapid recovery brought about by the administration of large doses of vitamin B would be very difficult to explain.

The work of Peters and his associates at Oxford University has contributed largely to the present understanding of the site and

nature of the injury due to a lack of the vitamin. Kinnersley and Peters<sup>1</sup> found an increase in lactic acid in the brain in pigeons showing head retraction, suggesting that the symptoms might be due to a failure of the oxidative mechanism in the brain or in a small part of it. Their later studies led to the conclusion that the vitamin is concerned with the oxidative removal of lactic and pyruvic acids. Further evidence for this viewpoint is furnished by the recent studies of Sherman and Elvehjem<sup>2</sup> who found a failure of pyruvate removal in vitamin B deficient chicks.

A specific effect of the accumulation of acid as a result of B deficiency is a slowing of the heart beat (bradycardia). This trouble is relieved by the removal of lactic acid which follows the ingestion of the vitamin.

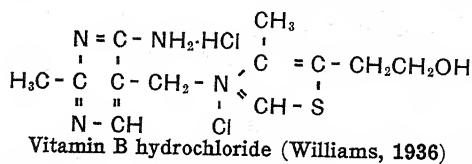
There is evidence that the symptoms of B deficiency develop more quickly on a fat-free diet and that fat exerts a sparing action on the vitamin requirement. A lack of vitamin B causes reproductive failure in both sexes. There is a larger requirement for lactation than for growth. Vitamin B is secreted in milk but never in large amounts. Apparently there is a normal value above which the level cannot be raised despite large intake in the feed, though this level may be lowered by rations poor in it (Sec. 334). The vitamin B content of eggs can be altered by the diet of the hen. The factor is stored in the body to only a limited extent.

**163. Chemistry of Vitamin B (B<sub>1</sub>).—**In 1926 the Dutch workers, Jansen and Donath, isolated vitamin B in crystalline form and reported an empirical formula containing carbon, hydrogen, oxygen, and nitrogen. Later Windhaus and coworkers prepared a crystalline product which was found to contain sulfur as well as the elements reported by Jansen and Donath. Other workers confirmed the presence of this additional element, and it was later found also in the crystals of the Dutch workers. Synthesis of the vitamin has very recently been accomplished by Williams and

<sup>1</sup> KINNERSLEY, H. W., and R. A. PETERS, Observations upon carbohydrate metabolism in birds. I. The relation between the lactic acid content of the brain and the symptoms of opisthotonus in rice-fed pigeons, *Biochem. J.*, **23**, 1126–1136, 1929. (See also later papers by these investigators.)

<sup>2</sup> SHERMAN, W. C., and C. A. ELVEHJEM, The effect of polyneuritis in chicks upon the in vivo rate of removal of pyruvate injected intravenously, *J. Nutrition*, **12**, 321–328, 1936.

Cline,<sup>1</sup> and Williams<sup>2</sup> has established the following structure:



Vitamin B is soluble in alcohol as well as water and is readily destroyed by heat, especially in the presence of alkali. In a dry state, it is stable at 100°C. for several hours, but moisture greatly accelerates the destruction, and thus it is much less stable to heat in fresh than in dry foods. Autoclaving destroys vitamin B, an observation which played an important role in the discovery that what was originally considered to be a single vitamin contains more than one factor.

**164. Vitamin B (B<sub>1</sub>) in Foods.**—Brewer's yeast is the richest known source of vitamin B. The factor is present in liberal amounts in a wide variety of foods. Whole cereal grains are rich sources. Since the vitamin is present primarily in the germ and seed coats, by-products containing the latter are richer than the whole kernel, while highly milled flour is very deficient. Wheat germ ranks next to yeast. Vegetables, particularly the leafy kinds, contain fair amounts. Liver, kidney, and egg yolk are rich animal products. Oranges rank highest among the fruits. The content in hays decreases as the plant matures and is less in the cured than in the fresh product. The content is correlated with leafiness, greenness, and protein content. In general, good quality hay is a substantial source, and, in a dry climate, there is practically no loss in storage. Milk is not a rich source and pasteurization for 30 min. at 145°F. destroys 25 per cent of its content according to Krauss and associates.<sup>3</sup> Commercial canning may cause a much greater destruction. There should be no concern about any lack of vitamin B in the rations of farm

<sup>1</sup> WILLIAMS, R. R., and J. K. CLINE, Synthesis of vitamin B<sub>1</sub>, *J. Am. Chem. Soc.*, **58**, 1504-1505, 1936.

<sup>2</sup> WILLIAMS, R. R., Structure of vitamin B<sub>1</sub>, *J. Am. Chem. Soc.*, **58**, 1063-1064, 1936.

<sup>3</sup> KRAUSS, W. E., J. H. ERS, and R. G. WASHBURN, Studies on the nutritive value of milk. II. The effect of pasteurization on some of the nutritive properties of milk, *Ohio Agr. Expt. Sta. Bull.* 518, 1933.

animals including poultry, but evidence is increasing that the human diet is not always adequately supplied even though evident symptoms may be absent. Children receiving almost exclusively a milk diet or milk and a highly milled cereal may not receive a sufficient amount. Some surprising improvements in growth have been obtained by additions to the diets of such children.

**165. Assay.**—Vitamin B is assayed by either a maintenance or curative test with pigeons or by a growth or curative test with rats. All methods are subject to certain criticisms, but they are of distinct value nevertheless. In the rat growth test, animals which have ceased to grow on a B-free diet are divided into (a) negative controls, (b) positive controls, (c) a group receiving graded amounts of the substance to be tested. The *Sherman unit* is the amount required daily to produce a gain of 3 g. per week over a period of four to eight weeks. The *international unit* proposed in 1934 is the vitamin B<sub>1</sub> content of 10 mg. of a standard material, fuller's earth, upon which vitamin B<sub>1</sub> from rice polishings has been absorbed.

#### FLAVIN FACTOR

Following the differentiation of the original vitamin B into two factors, vitamin G (B<sub>2</sub>) was considered to play a dual role in being essential for growth and also for the prevention of certain characteristic skin lesions referred to as dermatitis. Its role as an antidermatitis factor was first worked out with rats and later extended to include the prevention of human pellagra, black tongue in dogs and a pellagralike syndrome in chicks. Soon, however, sufficient data accumulated to make it clear that all of these effects were not due to a single factor, and, by 1935, a substantial amount of evidence was furnished that the factor primarily responsible for growth had little or nothing to do with the prevention of the characteristic dermatitis. Thus what was previously considered a single factor became the *vitamin G complex*. The recent findings have given rise to a difference of opinion as to which of the newly recognized factors shall bear the original name, vitamin G (B<sub>2</sub>). At the present time the majority of the investigators are using it to denote the growth factor. Fortunately this factor has been identified chemically as a *flavin* and thus it can be discussed under this name, leaving



it to the future to decide whether a letter designation shall be used.<sup>1</sup>

**166. Chemistry of Flavin.**—Milk was early recognized to be an excellent source of the original vitamin G, as measured by growth response. Observations that the growth effect exhibited by whey seemed to be associated with its greenish-yellow fluorescent pigment led to the isolation of this pigment, first called lactochrome and later *lactoflavin* (Fig. 18) as the biologically

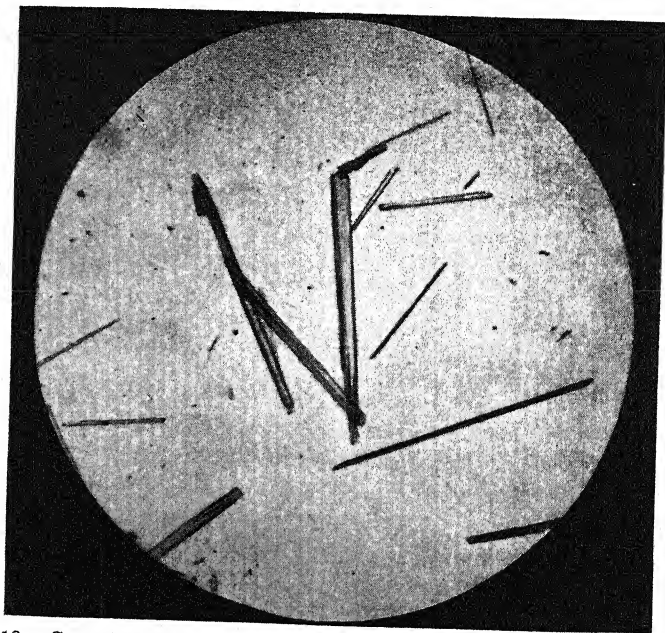


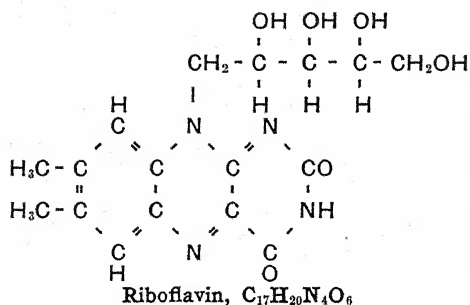
FIG. 18.—Crystalline lactoflavin. (Courtesy of George C. Supplee, The Dry Milk Company.)

active material. A similar compound was also isolated as hepato-flavin from liver and as ovoflavin from eggs. Later flavin was synthesized, and evidence was obtained for its identity with the naturally occurring products, both chemically and biologically. The identification of flavin as the growth-promoting principle of

<sup>1</sup> In the report of the committee of the American Society of Biological Chemists and the American Institute of Nutrition, approved by these societies in April, 1937, it was recommended that the letter designations for this vitamin be dropped and that it be called *riboflavin* in accordance with its chemical nature.



the vitamin G complex and the establishment of its chemical nature are due particularly to the brilliant work of György, Kuhn and associates in Germany, Karrer and coworkers in Switzerland, and von Euler and associates in Sweden. It is now accepted that the biologically active substance has the following structural formula:



The compound consists of an isoalloxazin nucleus combined with ribose as a side chain.

In liver and yeast, flavin occurs largely in a combined form. The work on flavin has led to the discovery that the yellow enzyme which Warburg found to be concerned in biological oxidations is a combination of the phosphoric-acid ester of flavin and protein. Flavin is heat stable in acid or neutral solution but readily destroyed in an alkaline medium. The autoclaving of yeast destroys its B<sub>1</sub> activity but not its growth-promoting power. Visible light, particularly the blue and violet rays, readily destroy flavin. Some investigators have reported that lactoflavin is not affected by pasteurization, while others have reported some destruction.

**167. Physiological Action of Flavin.**—In the absence of flavin from a diet adequate in all other known factors, a failure of growth and even a loss of weight results. Previous to the discovery that the vitamin G complex contained more than one factor, the production of cataract was reported by Day and Langston<sup>1</sup> as a characteristic finding in rats with diets deficient in this complex. Later work by Day and associates has proved that this injury is due to a lack of the flavin factor. Cataracts of a different

<sup>1</sup> DAY, PAUL L., and W. C. LANGSTON, Further experiments with cataract in albino rats resulting from the withdrawal of vitamin G (B<sub>2</sub>) from the diet, *J. Nutrition*, 7, 97-106, 1934.

nature have also been produced in rats by lactose-rich and galactose-containing diets.

In the poultry ration, flavin is essential for the production of eggs that will hatch, and the flavin content of the eggs varies with its content in the ration. Though it is agreed that flavin does not protect against the dermatitis which occurred in rats and chicks in the absence of vitamin G complex, certain workers have reported that a deficiency of flavin is responsible for a certain skin effect characterized by a shedding of the hair. While the need of flavin by mammals other than the rat and mouse has not been specifically shown, its requirement by other species seems probable.

**168. Distribution of Flavin in Foods.**—It is probable that the distribution of the original vitamin G complex, as measured by growth response, holds for flavin. This has been found to be true in so far as the foods have been specifically examined for flavin content. A fluorometric method for the estimation of lactoflavin has been devised. Liver, kidney, yeast, and milk, specifically the skim milk or whey fraction, are very rich sources of flavin. Leafy materials, fresh or dried, also contain important amounts. Meat and fish by-products used for animal feeding have a rather small and variable supply. Though seeds and their by-products show very low values, they provide appreciable amounts in commonly fed rations because of the large proportions in which they are included. In studies with rats and chicks, Hunt and coworkers<sup>1</sup> found that the content of the factor in the vitamin G complex measured by growth decreased in plant material with advancing maturity and that it was directly correlated with greenness, leafiness, and protein content.

A very recent report by Norris and coworkers<sup>2</sup> reviews the literature dealing with the identification of the growth principle of the vitamin G complex as a separate factor and presents data on its quantitative requirements by poultry and its distribution in commonly used feeds.

<sup>1</sup> HUNT, CHAS. H., P. R. RECORD, and R. M. BETHKE, Effect of the stage of maturity and method of curing upon the vitamin B and vitamin G content of alfalfa, clover, and timothy hays, *J. Agr. Research*, **51**, 251-258, 1935.

<sup>2</sup> NORRIS, L. C., H. S. WILGUS, JR., A. T. RINGROSE, VICTOR HEIMAN, and G. F. HEUSER. The vitamin-G requirement of poultry, *Cornell Agr. Expt. Sta. Bull.* 660, 1936.

## DERMATITIS FACTORS

The dermatitis in rats and chicks which was noted in the absences of the vitamin G complex is characterized in the rat by swelling and inflammation. In the chick there is an exudate of serum and scablike lesions around the mouth and eyes and on the feet. It is still agreed that this condition is caused by a dietary deficiency, but it now appears that more than one factor is involved. It is accepted that B<sub>6</sub>, first described by György, is a factor, different from the other recognized vitamins, which is essential to protect against this dermatitis in rats. There apparently is another factor which is essential to prevent the dermatitis in chicks. In fact, the several factors thus far announced by different workers suggest that not only does the dermatitis differ in the two species but also that there may be different kinds in the same species. The present situation with respect to these factors must be clarified by further experimentation before any extended discussion of them is worth while. Richardson and Hogan<sup>1</sup> in a report of their recent studies on the vitamin B complex have tabulated the properties which have been ascribed to seven rat-antidermatitis factors reported by different investigators and have presented a critical discussion of them.

**169. Pellagra.**—Our knowledge of the human disease, pellagra, which is characterized by a dermatitis, is due particularly to the work of Goldberger of the United States Public Health Service. In 1920 he showed that it resulted from the consumption of an ill-balanced diet, and in 1925 he established significant resemblances between the picture presented by pellagra in man, black tongue in dogs, and the skin lesions of rats caused by the lack of a factor in the vitamin B complex. His later work apparently led him to believe that human pellagra was a specific deficiency disease, caused by a lack of what was later designated as vitamin G. This opinion was increasingly held by others until the multiple nature of this factor was established. It now appears that flavin is not concerned in human pellagra, and it remains uncertain whether any of the antidermatitis factors reported for the rat or chick are so concerned. Pellagra was responsible for

<sup>1</sup> RICHARDSON, LUTHER R., and ALBERT G. HOGAN, Skin lesions of the rat associated with the vitamin B complex, *Mo. Agr. Expt. Sta. Research Bull.* 241, 1936.

7000 deaths in the United States in 1930, and there are many times this number of sufferers at the present time. Many foods are known, which, as supplements to diets on which the trouble occurs, will prevent it, but the specific factor involved remains uncertain.

Any importance that antidermatitis factors may have in the rations of animals other than the rat, chick, dog, and man is entirely unknown.

### VITAMIN C

Despite the early recognition that scurvy could be prevented by certain foods and despite the extensive work of Holst and Frölich previously referred to, it was not until about 1918 that the existence of a specific antiscorbutic dietary factor was clearly recognized. The factor was named "water-soluble C" and later vitamin C. Probably the delay was due in part to the fact that neither the rat nor the pigeon, which were used almost exclusively in the early vitamin studies, is susceptible to scurvy. So far as we know today, the only species which require vitamin C in their diets are man, the monkey, and the guinea pig. This does not mean that the other animals do not have a physiological need for it. The bodies of all of them which have been examined have been found to contain the antiscorbutic factor, and thus the probable reason why they do not require it in their food is that they are able to synthesize it from some dietary constituent.

Few studies have been carried out with farm animals with the rigidly controlled diets used with rats and guinea pigs. The experiments which have been made, however, have generally failed to indicate any need for the antiscorbutic factor in their diets, and the nature of their rations in practice makes it clear that they cannot have any large requirement. Controlled experiments with negative findings have been carried out with pigs by Orr and Crichton,<sup>1</sup> with calves by Thurston, Eckles, and Palmer,<sup>2</sup> and with chickens by Hart and associates.<sup>3</sup> In the latter studies the ability of chicks to synthesize the vitamin was demonstrated

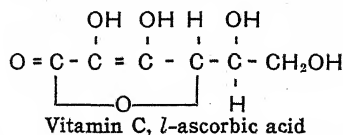
<sup>1</sup> ORR, J. B., and ARTHUR CRICHTON, The requirements of the pig for "vitamin A" and "vitamin C," *J. Agr. Sci.*, **14**, 114-125, 1924.

<sup>2</sup> THURSTON, L. M., C. H. ECKLES, and L. S. PALMER, The role of the antiscorbutic vitamin in the nutrition of calves, *J. Dairy Sci.*, **9**, 37-49, 1926.

<sup>3</sup> HART, E. B., HARRY STEENBOCK, and SAMUEL LEPKOVSKY, The nutritional requirement of the chicken. VI. Does the chicken require vitamin C? *J. Biol. Chem.*, **66**, 813-818, 1925.

by the fact that their livers were always rich in the factor despite its absence in the diet. It is also synthesized in the chick embryo. Madsen and coworkers<sup>1</sup> have raised sheep from weaning time to maturity on synthetic diets containing no source of vitamin C. No symptoms of scurvy were noted and when the animals were killed at nearly 500 days of age there were no gross or microscopic changes suggestive of this disease. These same workers made some incidental observations on the effect of adding ascorbic acid to rations for goats but no definite results were obtained.

**170. Chemistry of Vitamin C.**—The chemical goal of all vitamin research has been realized in the case of vitamin C in that it has been isolated, identified, and synthesized and is now available as a commercial product. In preparing concentrates from lemon juice, Zilva, of England, in 1925 noted that his most active fractions had strong reducing properties. In 1928 Szent-Gyorgyi isolated a hexuronic acid as a reducing principle from adrenal cortex and from certain plant tissues. He noted its similar distribution to vitamin C, but, at the time, no tests of the anti-scorbutic activity of the acid were carried out. In 1932 Waugh and King<sup>2</sup> of the University of Pittsburgh announced the isolation of a hexuronic acid from lemon juice which had marked anti-scorbutic properties, and, in the same year, Svirbely and Szent-Gyorgyi<sup>3</sup> reported similar properties for the product earlier isolated by the latter. As described by these workers the active product melts at 192°C. and has a specific rotation of +24°. In 1933 the structure of the compound was established through the work of Hirst, Haworth, and coworkers in England and by Reichstein and associates in Switzerland. The Swiss workers synthesized ascorbic acid and since the *l*-form was found to have an activity comparable to the natural product, the name, *l*-ascorbic acid, was proposed. It is known both by this name and also as cevitamic acid. The structural formula is as follows:



<sup>1</sup> MADSEN, McCAY, and MAYNARD, *loc. cit.*

<sup>2</sup> WAUGH, W. A., and C. G. KING, Isolation and identification of vitamin C, *J. Biol. Chem.*, **97**, 325-331, 1932.

<sup>3</sup> SVIRBELY, J. L., and ALBERT SZENT-GYORGYI, The chemical nature of vitamin C, *Biochem. J.*, **26**, 865-870, 1932.

Lemon juice yields from 100 to 150 mg. of ascorbic acid per liter as an isolation product. Paprika and pimento peppers are also rich sources.

The most characteristic chemical property of vitamin C is its reducing action which means that it is easily destroyed by oxidation. This action takes place most readily in an alkaline medium. Heat hastens its oxidative destruction, but, in an inert atmosphere, it is very stable to moderate heat. The reducing action of ascorbic acid provides the basis for the quantitative chemical determination of vitamin C in foods and tissues.

**171. Physiological Effects of Vitamin C.**—The clinical symptoms of scurvy are red blotches on the skin, bleeding gums, loosened teeth, swollen joints, brittle bones, and sensitiveness. These symptoms are caused by the occurrence of hemorrhage throughout the body which is the underlying pathological change and which may be responsible for a loss of energy, fleeting pain, and other signs of ill-health before the more characteristic symptoms appear. The hemorrhages are due to a weakening of the capillary walls throughout the body. Hemorrhages in the bones are responsible for the swollen joints, enlarged costochondral junctions, and the brittleness which results in easy fractures. The histological picture in the bones is quite different from rickets. The teeth are early affected, involving at the outset an injury to the odontoblasts. Serious damage may be done to the teeth before other symptoms are apparent, and vitamin C has come to be regarded by many workers as an important factor in preserving the integrity of the teeth and in preventing their decay. Hemorrhages in the brain and spinal cord are responsible for nervous symptoms.

By both biological and chemical methods, it has been shown that ascorbic acid is stored in the body, principally in the adrenal cortex. It occurs in only small amounts in the blood. It is secreted in milk but not in large amounts (Sec. 336). The vitamin found in the urine and the amount here excreted by species which cannot synthesize it are governed by both the state of the reserves and the current intake. The chemical determination of the ascorbic acid in the urine is a useful method of estimating the body's nutritive status with respect to it. A capillary-resistance test has also been developed for the same purpose. Further studies are required to determine its usefulness.

The specific mode of action of vitamin C, which is probably associated with its unique reducing properties, remains unknown. It has been found that ascorbic acid can activate or inhibit certain enzyme systems, and some studies have suggested that it may be concerned in cell respiration. An anti-infective role has also been ascribed to the vitamin.

Ascorbic acid is not present in resting seeds but is produced in them during germination. Some carbohydrate is doubtless the precursor, and, of the various sugars added to a culture solution, mannose has produced the largest amount of the vitamin. The question as to how and where ascorbic acid is synthesized in the body of species which do not require it in their food cannot be answered. Here again its precursor is probably a carbohydrate. It has been definitely shown that the adrenals, though they always contain considerable amounts of the vitamin, are not the site of its synthesis. There is some evidence that this synthesis takes place in the walls of the small intestine.

**172. Assay of Vitamin C.**—In the biological method of assay, guinea pigs are employed, using either a preventive or curative test. In the preventive technique, designed by Sherman and coworkers,<sup>1</sup> the animals are fed in a preliminary period a basal diet free from vitamin C plus some leafy material rich in it. The experimental period follows in which some of the animals, as negative controls, receive the basal diet alone, while others are fed the basal diet plus graduated amounts of the food to be tested. The amount of the test food which is just sufficient to protect the guinea pig from scurvy is called the minimum protective dose. *Sherman's unit* is the amount which as a daily dose just protects a 250- to 300-g. animal. Such protection is afforded by 0.5 milligram of ascorbic acid. In the curative procedure, the test substance is given in graded amounts following a period on a vitamin C free diet. This method has proved less satisfactory than the preventive one.

Ascorbic acid can be determined quantitatively by titration methods which take advantage of its reducing properties. The method in largest use at the present time is based upon the reaction of ascorbic acid with Tillmans' indicator, 2,6-dichloro-

<sup>1</sup> SHERMAN, H. C., V. K. LAMER, and H. L. CAMPBELL, The quantitative determination of the antiscorbutic vitamin (Vitamin C), *J. Am. Chem. Soc.*, **44**, 165-172, 1922.



phenolindophenol. Different procedures are employed by different investigators. The usefulness of these procedures for the assay of food materials is dependent on the extent to which interfering substances can be eliminated. For lemon, orange, and grapefruit juices, the results obtained with the dye method are in close agreement with those obtained by biological assay. With certain fruit and vegetable juices, however, there are interfering substances reacting with the dye which cannot be eliminated and which, therefore, cause inaccurate results. The chemical methods are generally useful in obtaining an approximate idea of ascorbic-acid content even where interfering substances are present, as providing a guide to the levels that should be used in a biological assay. In view of the great saving in time and money by the use of the chemical methods, they have proved of great value in extending the studies of the vitamin C content of various foods and in studying its metabolism. The method of Bessey and King,<sup>1</sup> designed for use with both plant and animal tissues has proved useful in the hands of several investigators.

The *International unit* for vitamin C is 0.05 mg. of *l*-ascorbic acid. It is thus one-tenth of the Sherman unit.

**173. Vitamin C in Foods.**—Fresh fruits and vegetables are the principal sources of vitamin C. Oranges, tangerines, lemons and grapefruit are very rich. Among the vegetables, the leafy varieties, such as cabbage, lettuce, watercress, spinach, and parsley rank with the fruits. So does the tomato, and certain peppers have an even higher value. Many other fruits and vegetables have considerable amounts. There are marked differences among varieties. In view of the ease of oxidation of ascorbic acid, air-dried products retain little if any of the vitamin. Seeds, hays, fats, and sugars have none. Cooking always results in some loss and may result in complete destruction. Heat may accelerate this destruction, but it has been found possible by the exclusion of air to cook and can many fruits and vegetables with little loss of the vitamin. This is particularly true of acid products such as the tomato. Canned tomato juice has been found a reliable and especially rich source. The loss is always much greater in a neutral or alkaline medium than in an acid one.

<sup>1</sup> BESSEY, O. A., and C. G. KING, The distribution of vitamin C in plant and animal tissues, and its determination, *J. Biol. Chem.*, **103**, 687-698, 1933.



Plant tissues which contain vitamin C also contain an ascorbic-acid-oxidizing enzyme which is released when the cells are crushed and which causes a rapid destruction of the vitamin in minced tissues. This destruction is much slower in an acid medium.

Animal products are poor sources of vitamin C. Fresh raw meat and eggs may contain a little. Certain glands contain more than muscle meat. Milk cannot be relied upon to contain enough to protect the child against scurvy. Freshly drawn milk contains a fairly constant amount of the vitamin, but there is a variable loss on standing caused by the action of various destructive factors. It can be pasteurized in such a way as to retain most or all of its vitamin content, but, in commercial pasteurization, a large loss commonly occurs and thus the product generally has little, if any, antiscorbutic value. Further discussion of factors influencing the vitamin C content of milk is given later (Sec. 336).

#### OTHER FACTORS

Though they may not have won universal acceptance, there are certain other factors in addition to those previously discussed which should be mentioned because of the body of evidence which has been presented for them.

**174. Trout Factor H.**—In 1927 McCay applied for the first time the purified diet method to the study of the nutritive requirements of trout. Early in these studies, which have extended over a period of nearly ten years, he ascertained that this species requires for growth some substance, which he called factor H, present in fresh meat but not in dried or cooked meats. McCay showed that this essential nutritive substance was different from any of the vitamins known at the time his discovery was made, and there is no convincing evidence that any of the more recently announced vitamins are identical with it. A detailed account of the experiments, resulting in the discovery of factor H, is given by McCay and Dilley.<sup>1</sup> While there is no evidence that this factor is required by any species other than trout, its recognition serves to call attention to the fact, definitely established by the experience of many feeders, that certain carnivorous animals, notably fox and mink, must have some raw meat in their diet. Whether

<sup>1</sup> McCAY, C. M., and W. E. DILLEY, Factor H in the nutrition of trout, *Trans. Am. Fisheries Soc.*, **57**, 250-260, 1927.

or not it be factor H, these species apparently require some vitamin different from those previously discussed.

**175. Chick Antihemorrhagic Factor.**—The existence of a fat-soluble factor, different from vitamins A, D, or E, which is essential for the chick to prevent the development of hemorrhages caused by a failure of the blood to clot, has been announced by Dam and by Almquist and Stokstad. It has been referred to as vitamin K. The factor is present in the unsaponifiable fraction of various animal and vegetable fats, and alfalfa is an especially rich source. The chemical and physical properties of a concentrate of this factor have been described by Almquist.<sup>1</sup>

**176. Vitamin B<sub>4</sub>.**—In 1929 Reader<sup>2</sup> produced evidence for a new factor originally called vitamin B<sub>3</sub> and later *vitamin B<sub>4</sub>*, based on deficiency symptoms which resembled those of B<sub>1</sub> but which were different in certain respects. Later an active crystalline substance was isolated and found to be identical with adenine hydrochloride, but adenine itself proved to have no activity. Despite much further work in various laboratories, there is at the present time no general agreement as to whether B<sub>4</sub> is actually a separate factor or whether the symptoms described for it simply represent a chronic state of B<sub>1</sub> deficiency. Several workers believe that it is a separate factor essential for the chick.

Jukes<sup>3</sup> has published an excellent review of experiments dealing with new factors required by chicks.

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<sup>1</sup> ALMQUIST, H. J., Purification of the antihemorrhagic vitamin, *J. Biol. Chem.*, **114**, 241–245, 1936; Chemical and physical studies of the antihemorrhagic vitamin, *ibid.*, **117**, 517–523, 1937.

<sup>2</sup> READER, VERA, A second thermolabile water-soluble accessory factor necessary for the nutrition of the rat, *Biochem. J.*, **23**, 689–694, 1929.

<sup>3</sup> JUKES, THOMAS H.: Recent studies of vitamins required by chicks. *J. Nutrition*, **13**, 359–385, 1937.

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## CHAPTER IX

### FEEDING EXPERIMENTS. THE DETERMINATION OF DIGESTIBILITY

Our previous discussion has considered the different nutrients which are required by the animal body and the metabolic changes which they undergo in serving its various functions. A knowledge of the quantitative needs of the body for these nutrients and of the relative value of feeds as sources of them is the basis of scientific feeding, a knowledge which has been gained gradually by means of research and experience over many years. An understanding of the methods by which it has been attained, and which are still being employed to augment it, is essential for the student of nutrition.

#### FEEDING EXPERIMENTS

Trial and experience were the means by which the art of feeding animals was originally developed. With the establishment of specific agencies to augment this knowledge, such as the agricultural experiment station, the feeding-trial method naturally was adopted as the means by which current practices could be critically tested and improved with the aid of the underlying sciences. A feeding trial with the species in question still remains the most useful method of obtaining results which have a direct application to feeding practice. But the method has a broader usefulness than this, as is indicated by the fact that feeding experiments with laboratory animals provide fundamental data which find application in human nutrition as well as in the feeding of farm animals. Feeding trials take many forms, a fact which must be appreciated if the results are to be interpreted correctly.

**177. Comparative Feeding Trials.**—In its simplest form, a feeding trial is a record of the results produced in terms of growth, milk production, or other function from a given feed or ration. Two or more rations may be compared with each other on this

basis. Additional records as to the feed eaten provide a comparison of the relative amounts of the rations required to produce a unit of product, and, by the use of cost figures, the results may be put on a money basis. The records here obtained tell us nothing as to why one ration proved better than another, unless the poorer one was so unpalatable as to be little consumed or unless it caused absolute harm. As a further step in the interest of more specific information, individual feeds may be compared as a part of rations the other ingredients of which are held constant. Here is an example which shows that fish meal is a better protein supplement for hogs than linseed meal:

	Average daily gain, lb.	Feed required for 100 lb. gain, lb.
Ration 1:		
200 lb. corn.....		
100 lb. wheat middlings...		
75 lb. fish meal.....	1.2	390
Ration 2:		
200 lb. corn.....		
100 lb. wheat middlings...		
75 lb. linseed meal.....	0.7	440

This experiment gives us a specific answer as to the comparative over-all effect of these two feeds but it tells nothing as to why the fish meal was better. Was it due to the higher percentage of protein in the fish product or to a higher biological value of this protein? Was the large amount of calcium supplied by the fish meal in contrast to the very small amount present in the oil meal a factor, or did certain vitamins present in the one but not the other play a role?

It is important to know the specific nutritive quality which makes one feed better than another. For example, if the superiority of the fish-meal ration was due entirely to the extra calcium supplied, the addition of ground limestone to the linseed-meal ration would provide a cheaper method of getting the same results. The comparison of two feeds with respect to a specific nutrient such as calcium or protein requires that all other nutritive factors be held alike and adequate in the two rations. This can never be achieved absolutely, but feeding trials can be set up

in such a way as to give most of the specific information desired, as is illustrated in connection with later discussions.

**178. Feeding Trials with Laboratory Animals.**—Today many of the problems of nutrition are being studied with small animals, such as the rat. The processes of growth, reproduction, and lactation can be effectively investigated, and the value of various feeds for these various functions determined. The much smaller cost in terms of animals, feed, and labor, and the much shorter time involved for a given experiment, in view of the short life cycle of the laboratory animal, are important advantages. The influence of individual variability, a serious disturbing factor in large-animal experimentation, can be reduced to a minimum by the use of animals of similar genetic and nutritional history, by the employment of large numbers, and by close environmental control. Slaughter for chemical and histological examination, a desirable feature of many feeding trials, presents little difficulty with small animals, compared to the economic and other considerations involved in the case of farm animals.

The laboratory animal is thus highly useful for working out many of the fundamental principles of nutrition. The results obtained in feeding trials with the small animals, however, cannot be considered to have direct application to the various species of farm animals, because of the differences in physiology and other considerations. Even here studies with small animals serve as pilot experiments, by means of which much preliminary information can be obtained more quickly and at much less cost than with the large animals, and whereby it can be determined what ideas are of sufficient promise to justify the expense involved in giving them a final test with the large animals. The situation is analogous to that of an industry in which processes worked out in the laboratory are first tested on a semicommercial scale before being finally adopted. Of course, there are feeding problems which by their nature are susceptible to solution only by experiments with the farm animals themselves, but the animal industry owes much to experiments with the rat.

**179. The Purified-diet Method.**—An important feature of feeding trials which has been developed along with the use of laboratory animals is the employment of purified or synthetic diets. These diets consist of purified sources of the various nutrients. For example, protein is supplied as casein, carbo-



hydrates as starch and sucrose, fat as lard or some oil, minerals as chemically pure salts, and vitamins as concentrated sources of them. Such a diet makes it possible to include or withdraw a given nutrient without disturbing any of the other nutrient relations. The influence of different protein levels can be studied by including varying amounts of casein without any change in the rest of the ration, whereas the addition of the natural source of casein, *viz.*, milk, would introduce many variables because it contains all of the other nutrients as well. Another protein such as zein of corn can be substituted for the casein and the relative value of the two determined. By similar procedures, the other nutrients can be subjected to specific study.

The extensive use of the purified-diet method has been a development of the last 25 years, but the idea was conceived more than a century ago, at least. As reported in 1816, Magendie fed diets of pure sugar and of pure fat to dogs to ascertain whether or not nitrogen was required in the food. Before the middle of the last century Boussingault,<sup>1</sup> the famous French chemist, carried on nutrition studies with various species, involving the use of diets consisting in part of purified nutrients. As later attempts were made from time to time to use this method, the discouraging result occurred that the more completely the diet consisted of purified nutrients, the less satisfactory was the effect on the animal. It was this discouraging result, however, that led to the conclusion, toward the close of the century, that there were dietary essentials unknown to the chemist and thus led to the later discovery of the vitamins and other previously unappreciated nutritive factors. There followed the intensive application of the purified-diet method by McCollum, by Osborne and Mendel, and by others, as a result of which an increasing knowledge as to essential constituents of a purified diet and new discoveries as to nutritional requirements simultaneously developed.

<sup>1</sup> J. B. Boussingault (1802-1877) following a period of service as professor of chemistry at Lyons, France, founded the first Agricultural Experiment Station in 1836 at Pechelbronn. Here his pioneer studies on the nutrition of various species of animals extended over many years and became models for later investigators. Boussingault ranks as one of the foremost agricultural scientists of all time. His two-volume work, "*Économie Rurale*," published in 1843 and 1844, and dealing with soils, crops, and fertilizers as well as the nutrition of cattle, horses, hogs, and other animals, is highly worth-while reading for the modern student.

Thus the purified-diet method became responsible for much of our modern knowledge of nutrition, including the physiology of the vitamins, the establishment of differences in protein quality, and more exact information regarding many of the minerals. Studies of the role of an element needed by the body in small amounts can be effectively carried out only with basal diets which may be freed from it and to which it may be added in known amounts. This is only possible with purified diets because a diet cannot be prepared from natural foods which will be free from the element in question. The use of purified diets came to be known as the *biological method* for testing foods as to their content of vitamins (Sec. 147) and as to the quality of their protein (Sec. 86).

The ingredients of these diets cannot be considered pure in the absolute sense. Starch cannot be entirely freed from mineral elements without breaking down its structure, and similar limitations apply to other nutrients to a greater or less degree. For most studies, however, it is possible to prepare the ingredients sufficiently pure to enable the diet to serve its purpose adequately. Special difficulties arise in connection with those vitamins which have not yet been prepared in pure form and which therefore must be included as concentrates.

The use of the purified-diet method has thus far been limited almost entirely to the rat, in part because the cost of the ingredients of these diets limits their use with larger animals and in part because much further study is required to develop satisfactory diets for certain species. Several investigators have used purified diets successfully in pig experiments. Diets satisfactory for dogs over short periods have also been devised. Hogan and associates<sup>1</sup> have reported diets on which four successive generations of chicks were reared. Herbivorous animals present special difficulties because of the roughage factor. Madsen and associates<sup>2</sup> have made extensive studies with sheep, goats, guinea pigs, and rabbits. Their results were the most successful in the case

<sup>1</sup> HOGAN, A. G., R. V. BOUCHER, and H. L. KEMPSTER, Adequacy of simplified rations for the complete life cycle of the chick, *J. Nutrition*, 10, 535-547, 1935.

<sup>2</sup> MADSEN, LOUIS L., C. M. McCAY, and L. A. MAYNARD, Synthetic diets for Herbivora, with special reference to the toxicity of cod-liver oil, *Cornell Agr. Expt. Sta. Mem.* 178, 1935.

of sheep and goats, as is illustrated in Fig. 19. Considerable success with rabbits and guinea pigs has been reported by Hogan and Ritchie,<sup>1</sup> and a diet which appears entirely satisfactory for

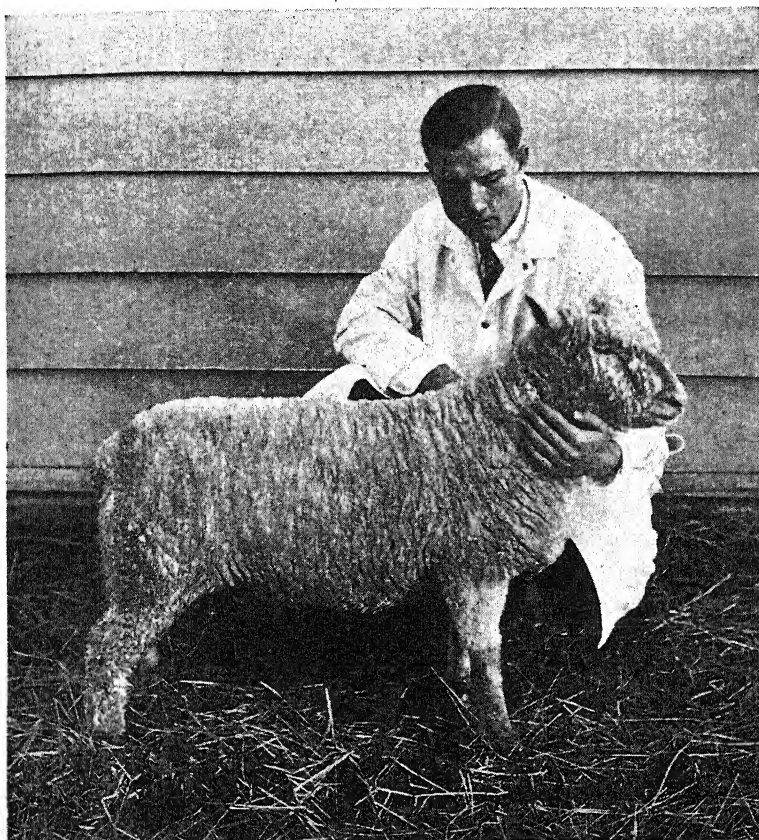


FIG. 19.—This sheep was reared from weaning to maturity on a synthetic diet which it received for 15 months.

guinea pigs has recently been described by Davis.<sup>2</sup> Further studies of purified diets for farm animals are needed, for there are

<sup>1</sup> HOGAN, ALBERT G., and WALTER S. RITCHIE, Nutritional requirements of rabbits and guinea pigs, *Mo. Agr. Expt. Sta. Research Bull.* 219, 1934.

<sup>2</sup> DAVIS, GEORGE KELSO, Further studies in the development of synthetic diets for Herbivora with special reference to cod-liver oil and cod-liver oil concentrates. A thesis presented to the Faculty of the Graduate School of Cornell University, 1937.

certain problems which cannot be satisfactorily solved until these diets can be successfully used.

**180. Group Feeding vs. Individual Feeding.**—Feed records are a desirable feature of all feeding trials. Even where the feed cost of the physiological performance is not of primary concern it is frequently essential from the standpoint of the interpretation of the results to have some record of the feed consumed. In most feeding experiments, particularly those with farm animals, the animals have been fed as a group. This is the simplest procedure from the standpoint of equipment needed and labor cost, but, in many experiments, it introduces complications in the interpretation of results. Such complications arise when there is a wide variability in the individual behavior within the lot, as to both production and feed consumption. The difficulty is increased when an animal owing to accident or other unavoidable cause has to be removed from the lot. The performance of the individual can be eliminated from consideration, but the food which it ate cannot. Individual feeding eliminates these disadvantages. It makes possible the correlation of individual performance record with the food which the animal ate. It preserves the identity of the individual. Certain species, which are fed together in practice, may consume somewhat less when fed individually.

While there are types of feeding experiments in which feed records of the group as a whole are sufficient or in which they may be acceptable in the interest of economy and of the use of larger numbers of animals, individual records are highly desirable in studies where only small differences are to be expected and where quantitative data are of special importance. Individual records are much more useful from the standpoint of statistical treatment (Sec. 185). The relative advantages of group and individual feeding are discussed in various papers presented in a symposium before the American Society of Animal Production.<sup>1</sup>

**181. Controlled Feed Intakes.**—Early in their studies of protein quality Osborne and Mendel recognized that ad libitum feeding frequently gave rise to variable results. They raised the

<sup>1</sup> CARROLL, W. E., Group feeding as a method of livestock experimentation, *Proc. Am. Soc. Animal Production*, 1930, pp. 34-44. LUSH, JAY L., Interpreting the results of group feeding experiments, *ibid.*, pp. 44-55. CRAMPTON, E. W., Individual feeding for the comparative feeding trial, *ibid.*, pp. 56-63.

fundamental question: "Does one animal grow because it eats more or the other fail because it eats less?" They experimented with various procedures of controlled feeding as a means of eliminating the uncertainties here involved. In one series of studies Osborne and Mendel<sup>1</sup> kept the food intakes alike for each diet under study, in accordance with a prescribed schedule based upon a preliminary experiment. They were thus able to compare the growth made on different diets consumed in the same amount. Recognizing that the more rapidly growing animals might be at a disadvantage under this system in view of their increasing maintenance requirement, they carried out another series in which the food intake was adjusted in accordance with increase in weight. In another experiment Osborne and Mendel<sup>2</sup> allowed ad libitum feeding and selected for comparison the growth records of those animals which had consumed substantially the same amount of food under this system. The discussion presented in these papers clearly shows that a proper assessment of the effect of food consumption as such is a very important matter in any feeding experiment, and the papers are well worth reading by any student who is planning such an experiment. This early work by Osborne and Mendel was a forerunner of the paired-feeding method which is widely used, but variously regarded, today.

**182. Paired Feeding.**—In this method of comparing two rations the animals are selected by pairs, one animal of a given pair being placed on ration A and the other being placed on ration B, and both animals being given exactly the same amount of food. The latter is accomplished by limiting the intakes of both to that of the animal consuming the lesser amount. The two animals of the pair are selected to be as nearly alike as possible in size, age, and previous history, but such equalities are not essential from pair to pair. The equalization of food intake is also limited to within the pair. This method is illustrated by the data presented in Table XIV, obtained in an experiment in which the two rations under comparison were alike with the exception of the

<sup>1</sup> OSBORNE, THOMAS B., and LAFAYETTE B. MENDEL, A quantitative comparison of casein, lactalbumin, and edestin for growth or maintenance, *J. Biol. Chem.*, **26**, 1-23, 1916.

<sup>2</sup> OSBORNE, THOMAS B., and LAFAYETTE B. MENDEL, The relative value of certain proteins and protein concentrates as supplements to corn gluten, *J. Biol. Chem.*, **29**, 69-92, 1917.

phosphorus carrier. Both rations contained the same amount of phosphorus and in the same ratio to calcium. It is noted that for a given pair of rats the food intakes were substantially alike over the experimental period of 35 days. When it is desired to compare three rations at the same time, the animals can be selected in trios. This may involve complications, however, in the equalization of food intake, complications which become increasingly troublesome in comparing more than three rations.

An important feature of a paired-feeding experiment is a statistical analysis of the data obtained, and the adaptability of the data to statistical treatment (Sec. 185) is a strong point in

TABLE XIV.—DATA FROM A PAIRED-FEEDING EXPERIMENT IN WHICH DICALCIUM PHOSPHATE (A) AND BONE MEAL (B) WERE COMPARED AS SOURCES OF PHOSPHORUS FOR BONE GROWTH<sup>1</sup>

	Pair 1		Pair 2		Pair 3		Pair 4	
	A	B	A	B	A	B	A	B
Food, g. ....	253	253	255	254	252	252	224	228
Ash in bone, %.	48.44	47.43	51.63	50.64	49.77	48.91	50.81	54.23
Ash in bone, mg.	191.0	157.7	190.3	154.7	179.2	166.3	162.3	171.3
Calcium in bone, %.....	19.81	16.04	18.59	18.20	17.78	17.63	18.20	19.26
Phosphorus in bone, %.....	9.9	8.5	9.3	9.2	8.9	8.8	9.0	9.6

<sup>1</sup> From ROTTENSTEN, K. V., and L. A. MAYNARD, The assimilation of phosphorus from dicalcium phosphate, C.P., tricalcium phosphate, C.P., bone dicalcium phosphate and cooked bonemeal, *J. Nutrition*, 8, 715-730, 1934.

favor of the method. Other things being equal, the larger the number of pairs the greater the reliability of the results. The data given in Table XIV include only four of the six pairs actually used in the experiment. The statistical analysis of the complete data revealed no certain advantage for one phosphorus carrier over the other.

In 1921 Armsby suggested the paired-feeding method as a procedure to be used in cooperative experiments to determine the protein requirements of calves. During the last 10 years, the method has been employed to study a wide variety of problems by Mitchell who is a strong champion of it. A paper by Mitchell



and Beadles<sup>1</sup> gives an excellent description of the procedure and its application. A unique advantage claimed for the paired-feeding method is that it eliminates the confusing effects which may arise from a variable food intake.

A criticism advanced against the method is that limiting the food intake may defeat the very object of the experiment, since a frequent effect of a nutritionally deficient ration is to decrease food consumption, with the result that the full effect of the better ration cannot express itself and that the comparison is made at a restricted food intake instead of a normal one. The force of this criticism is dependent upon the nature of the experiment. In answer to this criticism, the proponents of the method, though granting that it is not suitable to every type of problem, state that if a given ration is nutritionally superior to another its superiority should in general be evident when comparable animals receive the same intake, even though it is restricted. In practical tests in which palatability and level of food intake are important criteria of the relative value of the rations, equalized feeding would obviously defeat their purpose.

The method is not suitable for finding out *how much* superior one ration is to another, because, as the animal on the superior ration increases in weight over its mate, its maintenance requirement becomes greater than that of its mate. Under these conditions, an equal food intake for both means that the larger animal must be using a larger proportion for maintenance, and less remains for growth promotion. Therefore, an absolute equality of food intake means that the quantities available for the specific function which is being used as the criterion in comparing the two rations are not equal. The faster-growing animal is penalized. An attempt to overcome this limitation by adjusting the intakes in accordance with differences in maintenance requirements which develop is described by Maynard, Bender, and McCay.<sup>2</sup> In order to obtain quantitative data Mitchell and Hamilton<sup>3</sup>

<sup>1</sup> MITCHELL, H. H., and J. R. BEADLES, The paired-feeding method in nutrition experiments and its application to the problem of cystine deficiencies in food proteins, *J. Nutrition*, 2, 225-243, 1930.

<sup>2</sup> MAYNARD, L. A., R. C. BENDER, and C. M. MCCAY, Vitamin A and protein content of various fish meals, *J. Agr. Research*, 44, 591-603, 1932.

<sup>3</sup> MITCHELL, H. H., and T. S. HAMILTON, The balancing of rations with respect to protein, *Proc. Am. Soc. Animal Production*, 1935, pp. 241-252.

have fed pairs of rats amounts that would support equal gains in weight, using the differences in amounts fed as the measure of the relative nutritive value of the two rations under comparison.

The paired-feeding method seems sound in principle, but requires judgment in its use and in the interpretation of the results obtained. It would appear to find its largest usefulness in comparisons in which food consumption is not markedly restricted by the conditions imposed, and in which the measure is in terms of the specific effect of the nutrient under study, as is illustrated in Table XIV, instead of the more general measure of increase in weight. An excellent review of the use of the paired-feeding method, its applications, and limitations is given by Johnson, Hogan, and Ashworth.<sup>1</sup>

This discussion of paired-feeding illustrates the fact that no single method is suitable for the solution of all types of nutrition problems, that the effective investigator must select his method in accordance with his problem, frequently employing more than one method, and, finally, that he must interpret his results with a full consideration of the advantages and limitations of the method used.

**183. Slaughter Experiments.**—In the previously discussed experiment presented in Table XIV, the relative value of the two mineral supplements was measured in terms of the calcium and phosphorus content of the bones, since the growth of the animals as a whole would not have given definite information as to bone development. Such a procedure, which involves the killing of the animals and the analysis of certain specific tissues or of the body as a whole, is commonly referred to as a slaughter experiment. In many feeding trials, it is desirable to obtain more specific information regarding the effect of a given ration than is furnished by the common measures of weight and size. For example, in studies of the protein requirement for growth or of the comparative value of different protein sources, it is important to know the specific effect in terms of protein tissue formed since the increase in the body as a whole is due to water, fat, and minerals as well as protein, the relations of which may vary.

<sup>1</sup> JOHNSON, S. R., A. G. HOGAN, and U. S. ASHWORTH, The utilization of energy at different levels of protein intake, *Mo. Agr. Expt. Sta. Research Bull.* 246, 1936.



The introduction of the slaughter method by Lawes and Gilbert has been referred to. As now used it takes many forms according to the problem under investigation. To study the effect of a given diet on changes in body composition a group of like animals are selected and a part of them are slaughtered and analyzed at the start of the experiment. The others are fed a weighed and analyzed diet for a given period and then slaughtered and analyzed. The difference in their composition from that of the check animals killed at the start reveals the effect of the diet fed. The use of the slaughter method for studying protein and energy requirements is illustrated by the work of Mitchell and Hamilton.<sup>1</sup>

A slaughter experiment requires much more time and labor than is involved in merely weighing or measuring the animals, and in many instances difficult problems are presented in the selection of representative samples of tissues and in their preparation for analysis. For each period of observation, a sufficiently large number of animals must be examined to minimize the rather large individual variability in composition. In general, small laboratory animals are much easier to work with than the larger farm animals. Because of the economic considerations involved, work with the latter must be limited for the most part to those animals for which a return can be obtained on the carcass after the desired samples for analysis have been taken. As regards farm animals, therefore, the slaughter method has found its greatest application in studying the nutrition of beef cattle, sheep, and swine. Slaughter data may also include various measures of market value, such as dressing percentages and quality of the carcass, and such measures are frequently used in meat-production experiments to study the influence of a given ration upon the quality of the product and upon its selling price.

An excellent discussion of procedures for the handling of tissues obtained in slaughter experiments is presented in the report of the Subcommittee on Animal Nutrition of the National Research Council.<sup>2</sup>

<sup>1</sup> MITCHELL, H. H., and T. S. HAMILTON, Swine type studies. III. The energy and protein requirements of growing swine and the utilization of feed energy in growth, *Ill. Agr. Expt. Sta. Bull.* 323, 1929.

<sup>2</sup> FORBES, E. B., and H. S. GRINDLEY, On the formulation of methods of experimentation in animal production, *Nat. Research Council Bull.* 33, 1923.

**184. Financial Phases of Feeding Trials.**—It is obvious that an essential practical consideration in evaluating a ration for farm animals is its cost in terms of the return obtained for the product. Thus, in many feeding trials, records are kept of feed and perhaps of other costs, and of the estimated or actual selling price of the product. Profit or loss per animal or per unit of feed thus becomes a measure of the nutritive value of the ration. While it is obvious that the financial phases of feeding operations cannot be neglected, the expression of the results in terms of dollars and cents, unless properly interpreted, may obscure rather than clarify the facts brought out by the experiment. Monetary statements are not experimental results. They are based upon factors which are not under experimental control, the same combination of which may never occur again. The relative prices of feeds, and the selling price of the product vary from time to time, in fact from day to day and from place to place, according to market conditions. Clearly the product obtained per unit of feed is a much more stable and useful measure. While financial statements of feeding trials are interesting to the reader, they provide no basic or generalized measure of nutritive value and relying upon them as a guide for practice may prove disastrous. In contrast, a statement of food consumed and product obtained provides the basic data to which the feeder can apply current prices and thus obtain a much more accurate picture of the probable financial outcome than is given by a statement of the financial results based on prices at the time and under the conditions of the experiment.

**185. Statistical Methods of Analysis of Data from Nutrition Experiments.**—In a feeding trial certain factors, such as the amounts and quantities of feed, the time and method of feeding, and the general care and management, can be definitely fixed. Certain other factors, inherent in the animals used, cannot be controlled. The object of a well-planned experiment is to reduce these uncontrollable factors to a minimum by giving attention, in the selection of the animals used, to genetic and nutritional history as well as to such factors as age, size, vigor and the like. Even though this is effectively done there still remain inherent variables which cause two individuals to respond somewhat differently though treated exactly alike in an experiment. When treated differently, a part of the difference in response is due to the inherent variables and not to the treatment.

The effect of the inherent variables cannot be measured but the "probable error" in the experimental results caused by the uncontrollable variables can be estimated and taken into account. This is done by a statistical analysis of the data obtained, as has been incidentally referred to previously in this chapter. Such an analysis helps the investigator to decide whether the results obtained in a given comparison reflect a real difference in response to the two treatments, or whether they may have occurred simply because of inherent variations in the animals used. Statistical methods are also usefully employed in planning experiments in such a way as to make them most likely to give a definite answer to the question under study. They have become an essential tool of the investigator in nutrition, and some knowledge of them is helpful to all students in this field as an aid in the evaluation of published research. It is beyond the scope of this book to attempt any presentation of statistical methods, but, in the literature at the close of this chapter, several papers are cited which describe the procedures used and their usefulness in various types of research.

#### THE DETERMINATION OF DIGESTIBILITY

It is obviously impracticable to carry out feeding trials with all of the different feeds and combinations which are used in making rations; neither do these trials which measure only the final effect of the feed in terms of the function under study tell us much regarding the intermediate processes involved. There are other measures of nutritive value which give us more definite information as to why a particular result is obtained, and this information is useful both in experimental studies and in feeding practice. Chemical analysis is the starting point for determining the nutritive value of feeds, but the actual value of ingested nutrients is dependent upon the use which the body is able to make of them. The first consideration here is digestibility, since undigested nutrients do not get into the body proper.

Anatomical and physiological differences in the digestive tracts of various species are responsible for large variation among them in their ability to utilize different types of food in their nutrition. These variations are largest in the case of roughages, due to their content of complex polysaccharides (Sec. 42). None of the species of farm animals differ greatly in their ability to digest concentrates such as seeds and their by-products, since most of

these feeds are low in crude fiber. Fortunately, for experimental work, digestion in the rat is similar to that in the hog. The ability of the ruminant to handle a large amount of roughage gives it a special place in agriculture in that it can utilize the coarse products of the farm which find little outlet in other directions, whereas the hog competes more or less directly with man, or with the demands of industry, for most of its food. Owing to its capacious digestive tract, the ruminant can consume a ration which it only partially digests and still get enough nutrients for rapid growth or other function, whereas the hog must have a highly digestible ration in order to consume enough to meet its needs for best performance. Thus, for economic reasons, the rations fed to cows, sheep, and even horses are commonly those which are less digested than those fed to hogs.

**186. The Determination of Digestibility.**—A digestion trial involves a record of the nutrients consumed and of the amounts of them voided in the feces. It is essential that the feces collected represent quantitatively the undigested residue of the measured amount of food consumed. Various methods are employed for this fecal collection. In the case of Omnivora and Carnivora some indigestible, easily distinguishable substance called a *marker* may be used. The marker is fed just before the beginning of the ingestion of the ration to be tested, and again at its close. The feces collection is begun when the first marker appears and ended with the appearance of the second. A satisfactory marker must be inert physiologically and contain no element under investigation. The less the substance diffuses the better. Carmine is a frequently used marker. Ferric oxide, chromic oxide, and soot have also been employed. No marker can be considered to provide unquestionable accuracy.

In the case of herbivorous animals with their much larger and more complicated digestive tracts the use of a marker is not a suitable method. For these species, and commonly for other farm animals also, the ration to be tested is fed in constant daily amounts for an extended period. After allowing a certain number of days to elapse as a *preliminary* period to free the digestive tract of any indigestible material coming from the feed consumed prior to the start of the constant intakes of the ration under study, the collection of the feces is begun and continued through the *collection* period. The length of the periods required

to obtain reliable results depends upon the species, longer periods being necessary in the case of Herbivora, especially ruminants, than of other species. In general, the longer the period of collection, provided the stated amount of food continues to be consumed regularly and completely, the more accurate the results, since the effect of periodic fluctuations is minimized.

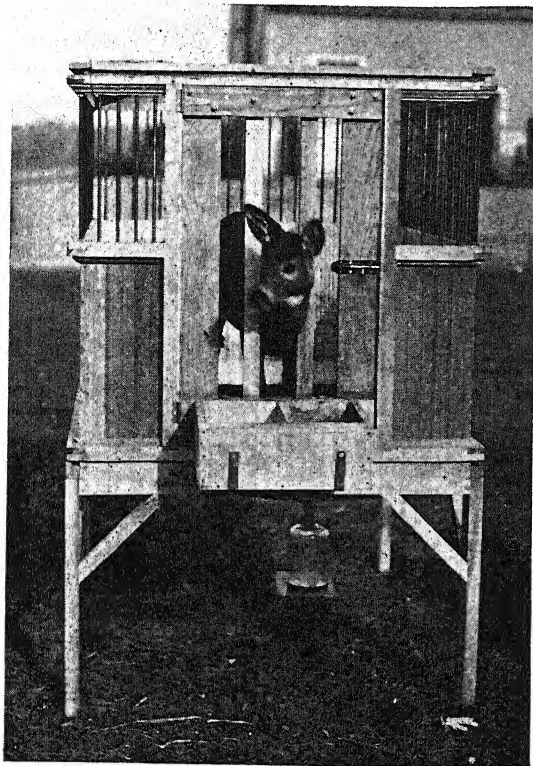


FIG. 20.—Metabolism cage for separation and collection of feces and urine. (From L. A. Maynard, Gardiner Bump, Robert Darrow, and J. C. Woodward, *Food preferences and requirements of the white-tailed deer*, N. Y. State Conservation Dept. & N. Y. State College of Agr. Bull. 1, 1935.)

**187. Methods of Collecting Feces.**—There are various methods used in the collection of the feces in a digestion trial. For laboratory animals, and also for sheep, hogs, and other animals of a similar size, metabolism cages have been devised which permit the accurate separation and collection of the excreta. Such a cage is shown in Fig. 20. The animal stands on an iron grid

through which the excreta pass. The feces are separated out by a suitable screen and the urine passes through into the bottle shown underneath. The feedbox is outside the cage, and the animal may be stanchioned during feeding. Thus no feed is scattered into the feces, and any scattered outside can be collected and accounted for.

Collection bags or similar devices which can be attached to the animal are commonly employed for steers and wethers. A bag designed by Garrigus and Rusk of the University of Illinois for

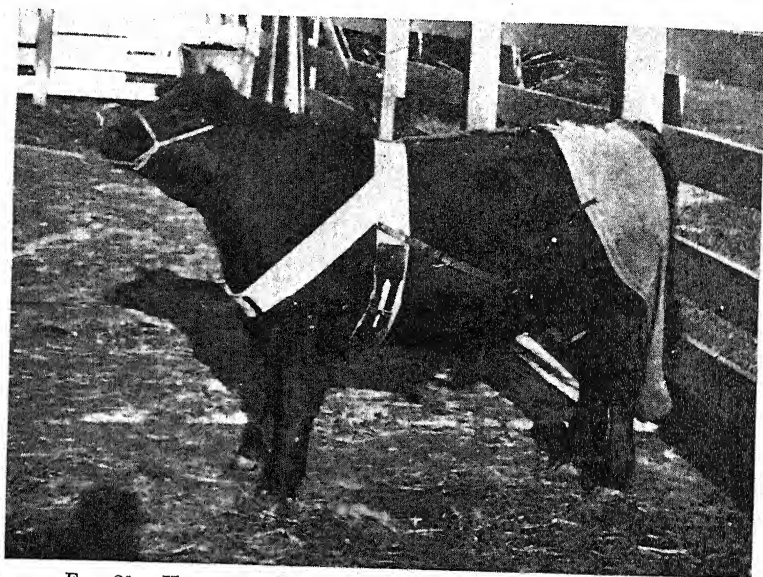


FIG. 21.—Harness and bag for collecting feces in a digestion trial.

collecting the feces of steers on pasture is shown in Fig. 21. Such a bag cannot be used for female animals because of the problem of separating the feces from the urine. With dairy cows the common procedure is to employ attendants who remain behind the animals constantly to collect the excreta as voided. This is an accurate but expensive method.

Ritzman and Colovos<sup>1</sup> have described a device which provides for the automatic collection and separation of the solid and liquid

<sup>1</sup> RITZMAN, E. G., and N. F. COLOVOS, An automatic method for collecting solid and liquid excreta from cows in digestion experiments, *N. H. Agr. Expt. Sta. Tech. Bull.* 52, 1932.



excreta of cows. The excreta as voided pass down a chute behind the cow and fall on a broad belt moving uphill. The urine drains off the belt into a collecting pan, whereas the feces remain on the belt and are dumped into a can as the belt goes over a roller. A simpler apparatus for use with cows which collects the urine and feces separately has been recently designed by Forbes and associates of the Institute of Animal Nutrition.

The determination of digestibility in poultry requires a special technique since the feces and urine are voided together, causing a mixing of the urinary and fecal nitrogen. The two forms of nitrogen can be separated by determining the ammonia and uric acid which represent the urinary output. The determination of digestibility is also carried out by the use of an operative technique which involves the formation of an artificial anus.

**188. A Digestion Trial.**—An example of the data obtained in a digestion trial is presented in Table XV. In obtaining the data for intake of nutrients, the feed intake was multiplied by figures for its percentage composition as determined by chemical analysis. Similarly, the data for excreted nutrients were calculated, and the digested nutrients obtained by subtraction. The final figures, expressed as percentages, are called *digestion coefficients*. In such a trial, several animals must be used and the results averaged to minimize the factor of individual variability.

It is noted that the mineral nutrients are not considered in Table XV. The discussion in Chap. VII has shown that some of the absorbed minerals are excreted through the gut. There is no method of separating such mineral material from the portion which originally failed of absorption. Since this is true and since the reexcreted portion may be a large as well as a variable part of the whole, it is impossible to arrive at a figure of any value for the digestibility of most of the mineral elements. Data for "digestible ash" which are frequently reported in connection with digestion trials have no real significance.

The digestibility of individual feeds may be determined in so far as they provide a satisfactory ration for the period of the test, when fed alone. The digestibility of concentrates by ruminants cannot be determined in this way because they do not provide sufficient bulk; their coefficients can be obtained only by difference. In this procedure the digestibility of a roughage as a basal ration is first determined, and then the concentrate is added to

the roughage for a second test. By a consideration of differences between the figures obtained for the roughage alone and for the combination, coefficients for the digestibility of the concentrate are calculated. Such figures represent the net effect of the addition of the concentrate to the roughage, but they may not be exact for the concentrate because its addition may have influenced the digestibility of the basal ration. The frequent occurrence of impossible coefficients in data obtained by the indirect method testifies to the fact that it is not always accurate.

TABLE XV.—DIGESTIBILITY OF DRIED GRASS BY A DAIRY COW<sup>1</sup>  
(Data for a One-week Period)

	Crude protein	Carbohydrates		Ether extract
		Fiber	N. F. E.	
Intake of 44,684 g. dry matter, containing, g.....	10,216	8,255	20,823	1,697
Output of 11,609 g. fecal dry matter, containing, g.....	2,559	2,158	4,042	783
Digested nutrients, g.....	7,657	6,097	16,781	914
Digested nutrients, %.....	75	73.9	80.6	53.9

<sup>1</sup> These data are taken from NEWLANDER, J. A., and C. H. JONES, The digestibility of artificially dried grass, *Vt. Agr. Expt., Sta. Bull.* 348, 1932.

**189. The Enzymatic Method of Determining Protein Digestibility.**—Waterman and associates designed a method for determining the digestibility of the proteins of foods in the laboratory by the use of a pepsin-hydrochloric-acid solution. This enzymatic method has proven useful for studying the relative digestibility of various sources of protein and for studying the influence of certain factors such as cooking on the digestion process. The method cannot be relied upon, however, to give the same results as are produced in vivo, for the conditions of the digestive tract cannot be duplicated in the test tube. This is particularly true for the roughages. When applied to hays and pasture grass, the enzymatic method has been found to give much higher values than are actually obtained with ruminants.

**190. Total Digestible Nutrients.**—As a general measure of the nutritive value of a feed, digestion coefficients are used to compute its content of total digestible nutrients. The dried grass used in



the digestion trial presented in Table XV had the following composition: crude protein, 20.11; crude fiber, 16.25; nitrogen-free extract, 40.99; ether extract, 3.34. The digestible nutrients are obtained from these data by multiplying them by the digestion coefficients given in the table, as is shown below:

Nutrient	Total nutrients in 100 lb., lb.	Digestion coefficients, per cent	Digestible nutrients, lb.
Crude protein.....	20.11	75	15.08
Crude fiber.....	16.25	73.9	12.01
Nitrogen-free extract.....	40.99	80.6	33.03
Ether extract.....	3.34	53.9 ( $\times 2.25$ )	4.04
Total digestible nutrients....			64.16

The digestible fat is multiplied by the factor 2.25, because it has that much more energy value than the other nutrients, as is explained later (Sec. 201).

Morrison<sup>1</sup> has compiled the data from digestion experiments carried out with the various feeding stuffs used for farm animals, and thus constructed a table of *average digestion coefficients*. He has applied these coefficients to figures which he has compiled on the average composition of these feeding stuffs and thus computed their content of digestible nutrients. These data, representing an exceedingly time-consuming and invaluable piece of work, are given in Table I in the appendix of his book. The values for digestible protein and total digestible nutrients are the ones commonly employed in the computation of rations for farm animals. The figures for concentrates can be considered to apply to all species. Those for roughages apply to cattle and sheep but are somewhat too high for the horse, and, of course, they do not hold for the pig. Morrison has compiled the available data on the digestibility of feeds by horses and swine and these data are also given in the appendix of his book.

**191. Nutritive Ratio.**—This is the ratio of the digestible protein, expressed as unity, to the sum of digestible carbohydrates

<sup>1</sup> MORRISON, F. B., Feeds and feeding, 20th ed., Morrison Publishing Company, Ithaca, N. Y.

and fat, the latter being multiplied by 2.25. The second factor of the ratio is calculated as follows:

$$\frac{(\text{digestible fat} \times 2.25) + \text{digestible N.F.E.} + \text{digestible fiber}}{\text{Digestible protein}}$$

Based upon the data previously given for dried grass (Sec. 190) this calculation results in the figure 3.2 and the ratio is therefore 1:3.2. Such a ratio is called a *narrow* one because of the relatively large amount of protein in relation to the other nutrients; where the reverse is true we have a *wide* ratio.

**192. Factors Affecting Digestibility.**—The influence of crude fiber content on digestibility has been discussed (Sec. 42). The extent of its protective effects in preventing access of the digestive juices to the other nutrients varies with the form in which a feed is fed as well as with the species. Even the starch grains have a protective cell membrane which limits the digestibility of raw starch by the young of all species. Cooking ruptures this membrane. The previous discussion of the digestibility of the carbohydrates also illustrates the fact that the nature of the nutrients themselves influences their digestibility. This is particularly true for the group comprising the ether extract. While in seeds, this extract consists almost entirely of readily digestible esters of fatty acids, this is not true for leafy material. Fraps and Rather<sup>1</sup> found in studies with eighteen different roughages that on the average only 42 per cent of the ether extract was saponifiable and that this fraction varied in digestibility from 8.6 to 92.3 per cent in the different plants, with an average of 66.4 per cent. The range of digestibility for the unsaponifiable fraction was 0 to 86.6 per cent, with an average of 29.1 per cent. Differences in digestibility have also been shown among proteins from different sources.

Digestibility may be limited by a lack of time for complete digestive action on less easily digestible substances or by a lack of complete absorption. Such an effect is heightened by a rapid passage of the food through the tract. On the other hand, food may move so slowly through the intestines as to be excessively subject to wasteful fermentations. Lack of time for digestion or

<sup>1</sup> FRAPS, G. S., and J. B. RATHER, Composition and digestibility of the ether extract of hays and fodders, *Texas Agr. Expt. Sta. Tech. Bull.* 150, 1912.

absorption may explain why, as the level of food intake increases above a certain value, the digestibility of all nutrients tends to decrease. Average digestion coefficients determined at or near maintenance are frequently found not to hold at the level of food intake required for liberal production. The early studies of Eckles<sup>1</sup> showing digestibility in the dairy cow to be lower at full feed than at maintenance have been confirmed by later workers.

As the nutritive ratio becomes wider, the digestibility of all nutrients tends to be lower. This is particularly true for protein, and the effect here is readily explainable on the basis of output of metabolic nitrogen, since the protein coefficient determined represents the apparent digestibility (Sec. 93). Inasmuch as the metabolic nitrogen is governed by total food intake and thus remains constant although the percentage of protein in the food is lowered, the fecal nitrogen as a whole does not decrease proportionally with the decreased protein intake, even though the residual food nitrogen may. Thus the apparent digestibility of protein is lowered with a wide ratio even though the true value may not be. Another explanation must be sought in the case of the other nutrients. It is possible that a modification in the bacterial flora, which alters the breakdown of the protective cellulose and related substances, is responsible. The lowering of the digestibility of nutrients, other than protein, with a ration having a wide nutritive ratio is less marked than for protein itself, and published data indicate that it occurs less consistently. It has been clearly demonstrated for cows as the work of Perkins and Monroe<sup>2</sup> testifies. It has also been shown in unpublished results of the Cornell workers which have led to the conclusion that a certain minimum level of protein intake for milk production is important not only to supply adequate protein but also in the interest of the digestibility of the ration as a whole.

It is recognized that the digestibility of a mixture is not necessarily the average of the values for its constituents determined separately or indirectly. Each feed may exert an influence on the digestibility of the others. Although it is impossible to

<sup>1</sup> ECKLES, C. H., Nutrients required for milk production, *Mo. Agr. Expt. Sta. Research Bull.* 7, 1913.

<sup>2</sup> PERKINS, A. E., and C. F. MONROE, Effect of high and low protein content on the digestibility and metabolism of dairy rations, *Ohio Agr. Expt. Sta. Bull.* 376, 1924.

determine the specific factors involved in this *associative digestibility*, the previous discussion of the influence of crude-fiber content and of nutritive ratio make it easy to understand that marked variations from computed averages may occur.

**193. Influence of Feed Preparation on Digestibility.**—Grinding grain usually does not increase digestibility in those animals which masticate their feed thoroughly, but seeds which escape mastication may remain largely undigested in passing through the tract. Sheep masticate their feed so effectively that there is no advantage in grinding grain for them, except in the case of very small and hard seeds. Cattle chew their grain less thoroughly and thus digest it somewhat better when it is ground. Grinding helps for very young animals before their teeth are developed and for old animals that have poor teeth. Digestibility in growing swine is only slightly increased by grinding, but the effect is more marked in older animals.

Differing from the case with grains, roughage is chewed by all animals sufficiently to break it up so that the digestive juices can penetrate it. There is no advantage in grinding or chopping hays which are of sufficiently good quality and palatability to be completely consumed without it. Grinding does, however, increase the consumption of coarse roughages and lessens the wastage which inevitably occurs when such roughages are fed uncut. This result is a distinct advantage in many feeding operations, but, with high-producing dairy cows where capacity to consume and digest food is a limiting factor, increasing the consumption of the less nutritious portion of the roughage may be definitely undesirable. It may be disadvantageous also for fattening calves.

Roughage should be coarsely ground or chopped rather than finely ground. The coarse product is more palatable and fine grinding may actually decrease digestibility. This has been shown to be true for alfalfa in studies with cattle at the Pennsylvania State College. A depression of rumination resulted which was the probable cause of the lower digestibility. From a practical standpoint, chopping or coarse grinding is preferable because it requires less power and is thus less expensive. Whether or not it pays to grind feeds under any conditions depends upon the gain in production relative to the cost of grinding.

Cooking feeds does not help digestibility in mature farm animals except in the case of a few feeds for swine, such as soy-

beans, field beans, and potatoes. The digestibility of soybean-oil meal by hogs is also improved by a thorough heat treatment, either in connection with oil removal or separately. Of course, cooking is destructive to certain vitamins. The newborn calf develops the ability to digest uncooked starch very rapidly. Shaw, Woodward, and Norton<sup>1</sup> found that its digestibility rose from a figure of 20 per cent at four to seven days of age to over 90 per cent at four weeks of age. None of the various processes of fermenting, "predigesting," and malting which have been exploited as methods for getting more nutrient value from roughages and other fibrous feeds have been found to have any advantage when subjected to critical tests.

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<sup>1</sup> SHAW, R. H., T. E. WOODWARD, and R. P. NORTON, Digestion of starch by the young calf, *J. Agr. Research*, **12**, 575-578, 1918.

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## CHAPTER X

### NUTRITIONAL BALANCES

The physiologists of four centuries ago, though they knew nothing about respiration, recognized that there must be some other loss from the body besides those in the feces and urine. They referred to this loss as the *insensible perspiration*, by which they meant the invisible exhalations which are known today as carbon dioxide and water. Sanctorius, a professor in the Medical School at Padua, who died in 1616, spent much of his life trying to measure this insensible loss by weighing himself, his food, and his excreta. An old print shows Sanctorius eating while seated in a chair balanced on a steelyard. He weighed himself before eating, added a weight corresponding to the amount of food he proposed to eat and stopped eating when his chair dipped.

In making these various measurements, Sanctorius performed what may be termed the first balance experiment. Such an experiment, as we know it today, involves a quantitative accounting for the intake of a given nutrient in the food and for its outgo in the excreta, providing data for determining whether there is a gain or loss of this nutrient by the body. Such an experiment constitutes another method of measuring nutritive value and the state of nutrition of the body. It gives specific information comparable to that of a slaughter experiment, previously described, and has the obvious advantage that it can be carried out with the living animal. Balance measures are commonly divided into two classes: those which deal with substances that can be weighed or measured, the *balance of matter*; and those which include heat losses, the *balance of energy*. A distinction between matter and energy is untenable according to modern physics, but it remains useful for the present discussion.

#### THE BALANCE OF MATTER

Boussingault<sup>1</sup> carried out the first real balance experiment in 1839. He measured the carbon, hydrogen, oxygen, nitrogen, and

<sup>1</sup> BOUSSINGAULT, J. B., *Analyses comparées des aliments consommés et des produits rendus par une vache laitière; recherches entreprises dans le*



ash in the food of a dairy cow receiving a ration that maintained her weight, and the outgo of these nutrients in the feces, urine, and milk. He recognized that he had not accounted for gaseous forms of the elements, and he used his data to estimate the atmospheric oxygen that was required by the cow. Later he made similar studies with a horse and other species. In our nutrition studies of today frequent use is made of the nitrogen balance, of various mineral balances, and, to a lesser extent, of the carbon balance.

**194. The Nitrogen Balance.**—A determination of the nitrogen in the food and excreta under controlled conditions provides a quantitative measure of the protein metabolism and specifically shows whether the body is gaining or losing protein. This is illustrated by the data in the following table, obtained with a steer receiving 8 lb. of clover hay daily:

Average daily nitrogen	Income, g.	Outgo, g.
In hay.....	71.4	
In feces.....	....	28.4
In urine.....	....	58.5
Lost from body.....	15.5	
Total.....	86.9	86.9

It is noted that the daily nitrogen intake was 15.5 g. less than the total outgo from the body and that the animal was thus in *negative nitrogen balance*. It was losing 96.9 g. ( $15.5 \times 6.25$ ) of protein from its body daily, representing the amount by which the intake of protein fell short of meeting the needs of the animal for maintenance. Had the nitrogen intake equaled the outgo, the animal would have been in *nitrogen equilibrium*, the normal picture in the mature animal which is receiving an intake of protein adequate for its needs. An excess of intake over outgo would have represented a *positive nitrogen balance*, involving a storage of protein in the body such as occurs in growth. Such a protein balance shows an increase in actual protein tissue, thus

but d'examiner si les animaux herbivores empruntent de l'azote à l'atmosphère, *Ann. chim. phys.*, 2d ser., 71, 113-127, 1839.



representing a more exact measure of growth than increase in weight which may be due in a varying degree to fattening.

The preceding example represents the simplest form of a nitrogen-balance experiment. The use of a ration consisting of several feeds may or may not require a record of the nitrogen intake from each, depending upon the feeding system and the objective sought. In a study of the protein metabolism in lactation, the nitrogen output in the milk must be accounted for. A determination of a nitrogen balance in a lactating cow shows whether the protein intake is adequate for the milk being produced or whether the milk protein is secreted in part at the expense of the body tissues of the animal. It is recognized that there are slight nitrogen losses in the skin excretions and in shed hair, and these are sometimes accounted for by analyzing the brushings from the animal. But this loss has been found so small that it is usually disregarded.

A nitrogen-balance experiment is carried out similarly to a digestion trial (Sec. 186) with the additional provision for the collection and analysis of the urine and of any product such as milk. For the determination of the balance, feces and urine can be collected together, and this is sometimes done since it is the simpler procedure and eliminates separate analyses. In most experiments, however, it is desired to know what part of the outgo is due to indigestibility and what part represents a loss in metabolism, and, for this purpose, the collections must be made separately by one of the methods previously described (Sec. 187). The nitrogen-balance method is used to determine the protein requirements for various body functions, to study the quality or biological value of the protein supplied by different feeds and rations, and for other purposes. These various uses are described later (Secs. 254, 255) and reference is made to papers which give detailed procedures.

**195. Mineral Balances.**—The balance of any mineral element can be obtained in the same way as is done for nitrogen since the same paths of outgo are involved. In fact, balance studies of nitrogen and of several mineral elements are frequently carried out together in the same experiment. The most frequently determined mineral balances are those for calcium and phosphorus. They provide an accurate measure of bone development during growth, and of the adequacy of calcium and phos-

phorus nutrition for various body functions such as maintenance, pregnancy, and lactation. Their use is described later (Sec. 267).

**196. The Measurement of Gaseous Exchange.**—In utilizing the balance method to determine the gain or loss of fat in the body and to study the value of any nutrient or ration for the production of energy, it is necessary to ascertain the intake and outgo of carbon. Since this element is eliminated in part in gaseous form, an accounting for such losses becomes essential. The amount of carbon lost through the lungs in respiration is obtained by a measurement of gaseous exchange, involving a determination of the oxygen consumed as well as the carbon dioxide eliminated. This measurement is an important feature also of the determination of the energy metabolism of the body since the production of energy is an oxidative process which involves the intake of oxygen, and the output of carbon dioxide and water. It is to Lavoisier that we owe the discovery of the true significance of respiration<sup>1</sup> as an oxidation comparable to combustion outside the body.

The determination of gaseous exchange can be carried out either by placing the subject in a chamber, the atmosphere of which can be controlled and measured, or by the use of a facepiece which provides for the analysis of the inspired and expired air. The use of the chamber makes possible an accounting for the water lost as perspiration and for the intestinal gases produced (Sec. 41), as well as the pulmonary exchange. These gaseous losses resulting from fermentations are of sufficient magnitude in Herbivora, especially ruminants, to require that they be determined or calculated in arriving at a carbon or energy balance. The various devices which are used in either of these methods are referred to as *respiration apparatus*. The earliest forms consisted of closed chambers in which the subject was placed and in which the change in the composition of the air was determined. The limitation of this procedure, which failed to provide for any renewal of the air or removal of waste products during the course of the experiment, is obvious. Two types of apparatus were later devised to remedy this defect; the *closed-circuit type* designed by Regnault and Reiset and the *open-circuit type* developed by Pettenkofer.

<sup>1</sup> See footnote, p. 8.

The closed-circuit type derived its name from the fact that the same air is continuously circulated, with provision for the removal of the waste products and the addition of oxygen. This apparatus is illustrated diagrammatically in Fig. 22. It is noted that the carbon dioxide and water are removed from the outgoing current by absorbents. Their output is determined by recording the increase in weight of the absorbing vessels. The oxygen of the circulating air is renewed through a meter by means of which the volume added is recorded. The residual air at the close of the experiment is analyzed to take account of any changes in composition from that at the start. In this apparatus, the intestinal carbon dioxide is absorbed along with that from the lungs. The

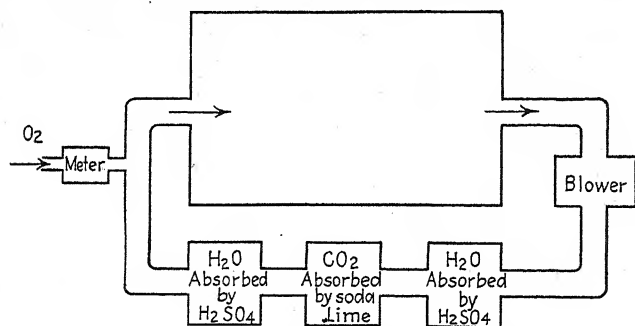


FIG. 22.—Closed-circuit respiration apparatus.

other intestinal gases, chiefly methane, can be determined in the residual air. Methane is determined by drawing the air sample over platinized kaolin, or a similar substance, at red heat. The methane is thus oxidized and determined from the carbon dioxide produced. Methane and other oxidizable gases present are thus referred to as combustible gases, a term which has special significance in connection with the energy balance (Sec. 206). Regnault and Reiset used their apparatus for studies with sheep, calves, hogs, and poultry, and the same principle is employed in the apparatus designed for man by Atwater and Benedict,<sup>1</sup> but

<sup>1</sup> William Olin Atwater (1844–1907) served for 34 years as professor of chemistry at Wesleyan University, Middletown, Conn. The first agricultural experiment station in the United States was established at Middletown under his direction in 1875. It was later moved to New Haven. Atwater also served as the first chief of the Office of Experiment Stations of the U. S. Department of Agriculture. In 1892, with the assistance of E. B. Rosa, professor of physics at Wesleyan, Atwater began the construction of the

the larger the animal the greater the difficulty and cost of constructing an airtight unit in which the temperature and humidity are well defined.

The open-circuit type differs from the one just described in that the circulating air is drawn from the atmosphere, and the outgoing air, or a measured fraction of it, is passed through the absorbents. When it is desired to account for the intestinal gases other than carbon dioxide, provision for their determination in the outgoing air as well as in the residual air of the chamber must be made. The Pettenkofer apparatus, originally designed for studies with men, was adapted for use with farm animals by later German workers, a development with which the names of Henneberg and Stohman, Kuhn, and later Kellner are especially associated.

In either the closed- or open-circuit type the chamber can be replaced by a facepiece or some other device for determining the pulmonary exchange only. Zuntz modified the Pettenkofer apparatus for use with the horse by eliminating the chamber and collecting the expired air by a tracheal canula, thus providing a portable device. Brody<sup>1</sup> has described an airtight muzzle for farm animals. When it is not desired to determine the losses in intestinal gases and in perspiration, the elimination of the chamber with its large volume of air has certain advantages, since short-time observations are thereby accurate and since changes can be traced sharply. For studies extending over several hours or days, the chamber type works better.

Modern respiration apparatus for farm animals include provisions for feeding and for the collection of the feces and urine, as well as for the determination of oxygen intake and of gaseous losses. They are described later (Sec. 209).

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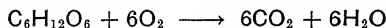
first human-respiration calorimeter which he later employed in his pioneer studies of heat production in man, of energy requirements for various body functions and of the nutritive value of foods. In this work Francis Gano Benedict early became associated. Upon Atwater's death, the calorimeter was moved to Boston and set up in the Nutrition Laboratory of the Carnegie Institution of Washington, where for 30 years Benedict and his associates have carried on outstanding studies of the energy metabolism in man and in various species of animals.

<sup>1</sup> BRODY, SAMUEL, Growth and development. XV. Energy and nitrogen metabolism during the first year of postnatal life, *Mo. Agr. Expt. Sta. Research Bull.* 143, 1930.

**197. Respiratory Quotient.**—The relation between the oxygen consumed and the carbon dioxide given off in respiration is expressed as the respiratory quotient, computed as follows:

$$\frac{\text{Volume of CO}_2 \text{ produced}}{\text{Volume of O}_2 \text{ consumed}} = \text{respiratory quotient (R.Q.)}$$

The numerical value of this quotient is dependent upon the chemical nature of the substance being oxidized within the body. The burning of a molecule of glucose, the form in which carbohydrates are catabolized, takes place according to the following equation:



Since the carbohydrate molecule contains hydrogen and oxygen in the proportion to form water, oxygen from the outside is required only for the oxidation of the carbon. One molecule of carbon dioxide is formed for each molecule of oxygen consumed, and, therefore, the respiratory quotient is 1.0. The fat molecule, on the other hand, does not contain nearly enough oxygen to take care of the hydrogen present, and thus a part of the oxygen used in burning fats appears as water. More oxygen is consumed, therefore, than is represented by the carbon dioxide given off and the R.Q. becomes less than 1.0. For most body and food fats, it is approximately 0.7. Such is the case for triolein, for example, as shown by the following equation for its complete oxidation:



For protein the basis for computing the respiratory quotient is less certain because the mode of its utilization in the body varies, because it is incompletely oxidized in catabolism, and because different ones vary in composition. The average figure commonly given for the R.Q. of protein is 0.8.

The magnitude of the respiratory quotient gives an approximate idea of the kind of nutrient which is being burned in the body. The closer the quotient approaches unity, the larger is the proportion of carbohydrates being used, while values lying close to 0.7 indicate that fat predominates as the body fuel. The meaning of an intermediate value is less clear since the quotient for protein lies in between those for carbohydrate and fat, and since a determined quotient may represent the result of the burning of variable proportions of all three. Respiratory quotients larger than unity are sometimes obtained, and they are explain-

able either on the basis that carbon dioxide is temporarily being given off more rapidly than it is being produced or on the basis that carbohydrate is being converted into a fat in the body. The synthesis of oxygen-poor fats from the relatively oxygen-rich carbohydrates involves a liberation of carbon dioxide. Wierzuchowski and Ling<sup>1</sup> report quotients of 1.4 and higher in rapidly fattening hogs, and they cite a quotient of similar magnitude obtained by Benedict in the goose. On the other hand, respiratory quotients below 0.7 have been observed in fasting, particularly in hibernating animals, and they may be the result of the conversion of fat into carbohydrate. They also occur in diabetes. It is evident that gaseous exchange is not limited to the oxidation of carbohydrates, fats, and protein. These various considerations indicate that the respiratory quotient can have only a limited usefulness as an exact indicator of the nature of the substances being burned in the body, but it is an important measure for certain other purposes, as is discussed later.

**198. The Nitrogen-carbon Balance.**—When carried out in conjunction with the nitrogen balance which measures changes in protein content, a carbon balance provides the additional data needed to obtain the gain or loss of fat. In thus measuring the changes in protein and fat content, an approximately complete picture of the influence of food upon the organic composition of the body is obtainable since its carbohydrate content is so small. Determinations are made of the carbon and nitrogen in the food, feces, and excreta and of the carbon in the gaseous output. Recognizing the limitations of his determinations of digestibility as measures of feed value, Henneberg, prior to 1870, turned his attention to the nitrogen-carbon balance in farm animals, taking his cue from the work of Voit and associates with man. Later a respiration chamber for large animals was built at the Mockern Experiment Station under the direction of Gustav Kuhn, and studies were undertaken which were carried out for the most part by Kuhn's successor, O. Kellner.<sup>2</sup>

<sup>1</sup> WIERZUCHOWSKI, M., and S. M. LING, On fat production in a young hog, *J. Biol. Chem.*, **64**, 697-707, 1925.

<sup>2</sup> Oscar Kellner (1851-1911), following short periods of service in the agricultural experiment stations at Proskau and Hohenheim and an extended period as professor of agricultural chemistry at the University of Tokyo, became director of the experiment station at Mockern in 1893. Here, he

As an example of a nitrogen-carbon balance, the data from one of Kellner's experiments<sup>1</sup> are presented in a condensed form in Table XVI. It is noted that from the nitrogen balance the amount of carbon gained as protein is calculated and that this value subtracted from the total carbon gained gives a figure which represents that gained as fat, from which the amount of

TABLE XVI.—EXAMPLE OF A NITROGEN-CARBON BALANCE

Item	Nitrogen		Carbon	
	Intake, g.	Outgo, g.	Intake, g.	Outgo, g.
Feed.....	390.55	.....	5668.2	
Feces.....	.....	105.69	.....	1456.9
Urine.....	.....	263.76	.....	283.3
Gases.....	.....	.....	.....	3247.9
Gain to body.....	.....	21.10	.....	680.1
Total.....	390.55	390.55	5668.2	5668.2

Based upon a content of 52.54<sup>1</sup> per cent carbon and 16.67<sup>1</sup> per cent nitrogen in fat-free, ash-free flesh, and of 76.5 per cent carbon in fat, the following calculations gave the protein and fat gained:

21.1 g. nitrogen gain divided by 0.1667 equaled 126.6 g. protein gain.

126.6 g. protein times 0.5254 equaled 66.5 g. carbon in protein.

680.1 g. carbon gain minus 66.5 equaled 613.6 g. carbon gained as fat.

613.6 divided by 0.765 equaled 802.1 g. fat gain.

<sup>1</sup> Figures used by later workers are slightly different.

fat can be computed. The computation is based upon the fact that the carbon content of the body exists almost entirely as protein and fat. It disregards the small amount of glycogen which is normally present, since it is considered that any changes in this constituent are so small as to be of very minor importance

served until his death. His many accurately conducted respiration studies with farm animals made a large contribution to the fundamental knowledge of nutritional physiology and found practical application in his feeding standards. Kellner's textbook "Die Ernährung der landwirtschaftliche Nutztiere," the first edition of which was published in 1905, contains an extensive account of his respiration studies and describes his feeding standards.

<sup>1</sup> KELLNER, O., and A. KOHLER, Untersuchungen über den Stoff- und Energie-umsatz des erwachsenen Rindes der Erhaltungs- und Produktions-futter, *Landw. Vers. St.*, 53, 1-16, 1900.

under normal feeding conditions. This is less true when the diet is such as to cause a loss, rather than a storage, of fat. For experiments over an extended period, disregarding the glycogen is of no concern, but, in experiments of only a few hours' duration, a considerable error may be introduced. The glycogen changes can be estimated by including determinations of hydrogen and oxygen in the balance data, a procedure which makes the experiment much more difficult and laborious. The most important use of the carbon- and nitrogen-balance method today is in connection with indirect calorimetry (Sec. 208).

**199. Kellner's Starch Values.**—Using the nitrogen-carbon-balance method, Kellner added pure carbohydrate, protein, and fat to a basal-maintenance ration and thus determined the relative amounts of these pure digestible nutrients required to produce a unit of body fat. When he tested feeding stuffs instead of pure nutrients, he found that the fat-producing power was less than calculated from their content of digestible nutrients and that the discrepancy was larger with those feeds high in fiber. He concluded, therefore, that some of the calculated fat-producing power was lost as a result of the "work of digestion" which increased with fiber content. Having determined the actual fat-producing power of a number of typical feeds, he worked out factors for estimating the loss due to the work of digestion for feeds of varying fiber content and thus obtained a basis for computing fat-producing power for a given feed from its content of digestible nutrients. He did not express this fat-producing power of the feed directly but rather in terms of the number of kilos of starch that would be required to produce the same amount of fat as 100 kg. of the feed. Hence his values were called *starch equivalents* or *starch values*. For example, the starch value of corn meal, 81.5 kg., was the amount of starch which would produce as much fat as 100 kg. of this feed. In the appendix of his book, Kellner published such values for approximately 300 feeds, a few of which are listed below:

Oats.....	59.7
Wheat.....	71.3
Linseed-oil meal.....	71.8
Wheat bran.....	45.0
Timothy hay.....	29.1
Oat straw.....	17.0



Kellner's starch values became the basis of his feeding standard, and they are still used for evaluating feeds and computing rations for farm animals in most European countries. Further reference is made to them later (Chap. XI).

### THE BALANCE OF ENERGY

The largest purpose which food serves is the production of energy for body processes. Since all the organic nutrients can serve this purpose, energy value provides a common basis for expressing their nutritive value. The fact that all these nutrients, notably protein, may have specific and unique functions as well does not alter their common usefulness as sources of energy. This holds whether they are used for the purpose immediately upon absorption or whether they are built into body tissue; for the glycogen and fat of the body constitute reserves which can be used as needed, and, when these supplies are exhausted, the protein of structural tissues can be broken down to serve as energy. Thus a measure of the gain or loss of energy provides a useful measure of the state of nutrition of the body and of the relative value of various foods. It takes into account a further loss not measurable by the balances previously discussed, *viz.*, the loss of heat.

**200. The Unit of Energy Value.**—Since all forms of energy are convertible into heat, it has been found convenient to express the energy changes of body processes in terms of heat units (calories). In this connection it is important to realize that it is not heat itself which the body uses. The body is not a heat engine (Sec. 339). The *small calorie* is defined as the amount of heat required to raise 1 g. of water 1°C. The *large Calorie*, written with a capital C to distinguish it from the small calorie, is the amount required to raise 1 kg. of water 1°C. It is the one commonly used in expressing the energy value of foods. A *Therm* is 1000 Cal. and is used as a matter of convenience where large values are involved.

**201. Gross Energy, Heat of Combustion.**—When a substance is completely burned to its ultimate oxidation products, *viz.*, carbon dioxide, water, and other gases, the heat given off is considered as its gross energy or heat of combustion. This measure is the starting point in determining the energy value of

foods. The determination is carried out in a calorimeter, of which there are various types.

The *bomb calorimeter* consists essentially of a bomb in which the food is burned, enclosed in an insulated jacket containing water which surrounds the bomb and which thus provides the means of measuring the heat produced. The construction of the Parr

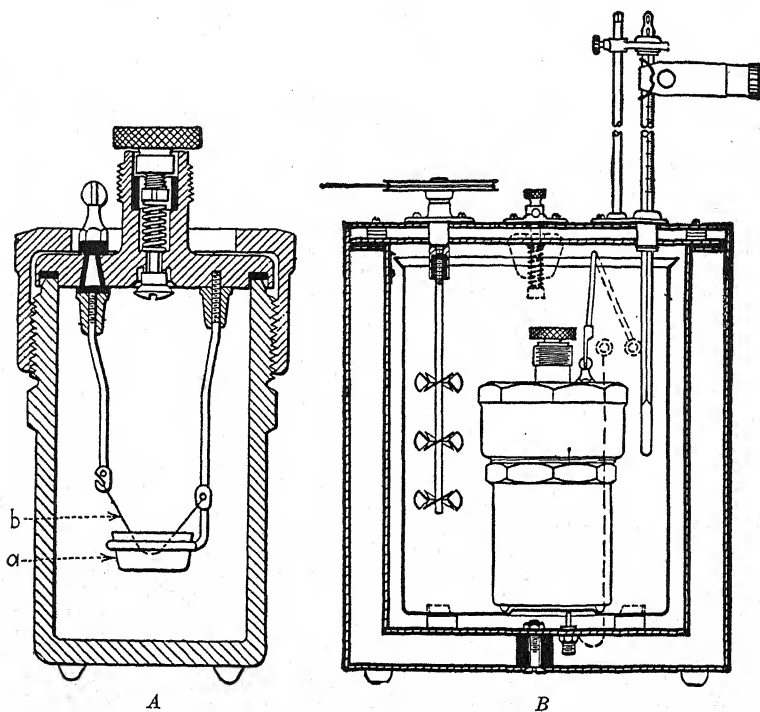


FIG. 23.—The Parr bomb calorimeter. A, cross section of bomb; B, cross section of calorimeter with bomb in place. (Reproduced by permission of the Parr Instrument Company, Moline, Ill.)

oxygen calorimeter is illustrated in Fig. 23. In A, the bomb is shown in cross section. A weighed amount of substance to be tested is placed in the cup *a* of the bomb, and the fuse wire *b* connecting the two terminals is put in place. The cover is screwed on and the bomb charged with 25 to 30 atmospheres of oxygen. The bomb is then placed in the calorimeter jacket as shown in B, surrounded by a known volume of water. The stirrer is started, and, when the temperature becomes constant, the charge is

ignited electrically and readings are taken on the thermometer to ascertain the maximum rise. This value multiplied by the sum of thermal capacity of the metal parts and the mass of water gives the number of calories produced by the burning of the sample. Various corrections are required for radiation, for the fuse wire, for acids produced and for other factors in arriving at the final figure for the calorific value of the food. The radiation corrections are eliminated in the *adiabatic type* of bomb calorimeter.

The *oxycalorimeter*, developed by Benedict and Fox,<sup>1</sup> measures the volume of oxygen required to burn a known weight of food. This value is used to calculate the heat of combustion by the use of factors established with the bomb calorimeter.

The gross energy values of some pure nutrients and feeding stuffs are given in Table XVII.

TABLE XVII.—GROSS ENERGY VALUES OR HEATS OF COMBUSTION  
(Dry matter basis)

Pure nutrients	Cal. per g.	Feeding stuffs	Cal. per g.
Glucose.....	3.76	Corn meal.....	4.43
Sucrose.....	3.96	Oats.....	4.68
Starch.....	4.23	Soybeans.....	5.52
Butter fat.....	9.21	Wheat bran.....	4.54
Lard.....	9.48	Linseed-oil meal.....	5.12
Seed fat.....	9.33	Timothy hay.....	4.51
Casein.....	5.86	Clover hay.....	4.47
Elastin.....	5.96	Corn stover.....	4.33
Gliadin.....	5.74	Oat straw.....	4.43

It is noted that among the pure nutrients the fats have approximately twice the energy value of the carbohydrates and that the proteins occupy an intermediate position. These differences are governed by elementary composition, especially the relative amount of oxygen contained in the molecule, since heat is produced only from the oxidation which results from the union with oxygen from without (Sec. 197). In the case of carbohydrates, there is enough oxygen present in the molecule to take

<sup>1</sup> BENEDICT, FRANCIS G., and EDWARD L. FOX, The oxy-calorimeter, *Ind. Eng. Chem.*, **17**, 912-918, 1925.

care of all the hydrogen present, and thus heat arises only from the oxidation of the carbon. In the case of fat, however, there is relatively much less oxygen present and relatively more atoms requiring oxygen from without, and the combustion involves the oxidation of hydrogen as well as carbon. The burning of 1 g. of hydrogen produces over four times as much heat as is the case for carbon. These facts explain the much greater gross energy values for the fats compared with the carbohydrates. The heat produced in the burning of protein comes from the oxidation of both carbon and hydrogen, but the nitrogen present gives rise to no heat at all because it is set free as such in its gaseous form. No oxidation of it has taken place, and thus no heat is produced.

These differences in elementary composition also explain the differences which occur among members of the three classes of nutrients. In both starch and glucose, for example, the hydrogen is taken care of by the oxygen in the molecule, but there is relatively more carbon in 1 g. of starch than in 1 g. of glucose, and thus starch has the higher energy value accordingly. The following values are commonly used as the average heats of combustion of the three classes of nutrients as they occur in foods:

Carbohydrates.....	4.1 Cal. per gram
Fats.....	9.45 Cal. per gram
Protein.....	5.65 Cal. per gram

These figures provide a basis for computing the gross energy values of foods from their chemical composition. They also serve to explain the differences in gross energy among various feed stuffs exemplified in Table XVII. Fat content is of special importance because of its high heat of combustion. The difference between the values for the soybean, an oil-bearing seed, and for the cereal grains which contain only about one-quarter as much fat, is illustrative of this.

**202. Metabolizable Energy.**—Not all of the gross energy as determined in the bomb calorimeter is useful to the body. A part of it escapes in the undigested portion and as combustible gases. Though the latter are readily burned giving rise to heat which is included in gross energy, their energy is not useful to the body. A further portion of the gross energy escapes unused due to the fact that the complete oxidation which gives rise to this energy does not take place in the body for all the absorbed

nutrients. Whereas the end products of protein oxidation in the calorimeter are carbon dioxide, water, and nitrogen, catabolism in the body results in urea and other incompletely oxidized nitrogenous end products which are excreted in the urine.

A determination of the heats of combustion of the feces and urine<sup>1</sup> and a measurement or calculation of the calorific value of any combustible gases produced in the digestive tract furnish data as to the part of the ingested gross energy which escapes from the body unused. The sum of these losses subtracted from the gross energy gives what is called *metabolizable energy*—the portion of the total which is actually capable of transformation within the body. There are small amounts of unused energy which escape as perspiration, epidermal scales, and shed hair. If accounted for, they should be subtracted in arriving at metabolizable energy, but they are so small that no significant error is ordinarily involved in neglecting them.

An illustration of the data involved in the determination of metabolizable energy is given in Table XVIII comprising figures obtained with sheep by Hamilton, Mitchell, and Kammlade.<sup>2</sup>

TABLE XVIII.—METABOLIZABLE ENERGY OF FEEDS FOR SHEEP

Feed	Dry matter eaten per day per head, kg.	Energy per kg. of dry matter, Therms				
		Intake in food	Losses			Metabolizable
			Feces	Urine	Methane	
Soybean.....	0.795	4.333	2.033	0.196	0.208	1.896
Soybean straw...	0.674	4.345	2.676	0.042	0.229	1.398

It is noted that by far the largest part of the losses is that which occurs in the feces as a result of lack of digestion. This illustrates the fact that metabolizable energy takes account of the same losses, for the most part, as does total digestible nutrients. It does represent a more accurate measure since further losses are accounted for. The much larger fecal loss for the straw is the

<sup>1</sup> The gross energy of urine can also be roughly calculated from its carbon content.

<sup>2</sup> HAMILTON, T. S., H. H. MITCHELL, and W. G. KAMMLADE, The digestibility and metabolizable energy of soybean products for sheep, *Ill. Agr. Expt. Sta. Bull.* 303, 1928.

primary factor causing its lower metabolizable energy, corresponding to its lower digestibility. The straw has a smaller urinary loss than does the hay because of its lower content of protein, the nutrient which is less completely oxidized in metabolism than in the bomb. The methane losses are similar for the two feeds.

**203. Physiological Fuel Values.**—These are calorific values for individual nutrients which are used in human nutrition to calculate the portion of the gross energy which is available for transformation in the body, a calculation which results in figures having a similar significance as metabolizable energy. Account is taken of losses in digestion on the basis of the following average figures for digestibility of a mixed diet: carbohydrates, 98 per cent; fats, 95 per cent; protein, 92 per cent. The figures for gross energy, corrected in the case of protein for the energy lost in the urine, are multiplied by these coefficients. Physiological fuel values are thus based upon the following calculations:

$$\text{Carbohydrates } 4.1 \times 98\% = 4 \text{ Cal. per gram}$$

$$\text{Fats } 9.45 \times 95\% = 9 \text{ Cal. per gram}$$

$$\text{Protein } (5.65 - 1.3) \times 92\% = 4 \text{ Cal. per gram}$$

The figure of 1.3 Calories is subtracted from the gross energy of protein because this is the part which escapes unburned in the urine. These values furnish the explanation as to why fat is multiplied by 2.25 in computing total digestible nutrients (Sec. 190).

The physiological fuel values shown above are the figures commonly applied to data for the chemical analysis of human foods to arrive at their fuel or calorific value as set forth in tables used in computing diets or comparing foods. The analysis of foods to provide data for such a table was one of the important contributions of Atwater.<sup>1</sup> Tables based largely on this original work of Atwater and including all the common human foods are published in the textbook by Sherman.<sup>2</sup>

The *law of isodynamic replacement*, as formulated by Rubner, states that the several nutrients can replace each other in amounts

<sup>1</sup> ATWATER, W. O., and A. P. BRYANT, The chemical composition of American food materials, *U. S. Dept. Agr. Office of Experiment Stations Bull.* 28, rev. ed., 1906.

<sup>2</sup> SHERMAN, H. C., Chemistry of foods and nutrition, 5th ed., The Macmillan Company, New York, 1937.

inversely proportional to their physiological fuel values. Its validity is recognized in the present practice of computing total digestible nutrients and in the use of the physiological fuel values for the computation of the calorific value of the foods and rations.

**204. Heat Production.**—In addition to the losses which are subtracted in obtaining metabolizable energy, the energy balance must take account of a further loss, *viz.*, that energy escaping in the form of heat. In every cell of a living organism, chemical reactions are constantly occurring as an essential accompaniment and manifestation of life processes. Most of these reactions are oxidative in nature producing heat. There results a continual outgo of heat from the body in amounts which represent a considerable fraction of the total income of chemical energy.

In all warm-blooded animals, the maintenance of a constant body temperature is a factor affecting heat production and heat outgo. Since the temperature of the body is normally above its environment, the heat constantly being produced serves in the maintenance of this temperature. The environmental temperature and the amount of heat being produced within the body are the factors which determine the extent to which this heat must be conserved. The amount which is allowed to escape from the body is subject to control which is referred to as *physical regulation*. This control is brought about by an adjustment of the blood flow to the skin and by the perspiration mechanism. If the conditions call for the dissipation of body heat, the blood flow to the surface is increased as a result of a dilation of the capillaries, which facilitates the escape of heat by radiation, and the pores are opened which allow for a loss of heat through evaporation. These processes are reversed when there is need for the conservation of body heat.

The effects of a low environmental temperature may be combated also by *chemical regulation*, involving the increased oxidation of body substance and resulting in an increased heat production. Shivering is an involuntary form of muscular activity the function of which appears to be to increase heat production when physical regulation proves insufficient. The environmental temperature at which physical regulation proves insufficient to maintain body temperature, and the point, therefore, at which chemical regulation must come into play, is called the *critical temperature*. As determined for the quiet-fasting condition, the

following values have been reported: rat, 28°C.; mouse 29 to 30°C.; chick, 17°C.; guinea pig, 32 to 33°C.; rabbit, 27 to 28°C.; hog, 21°C.; man, 15°C. These values are modified by various factors. Removing the hair coat, as in shearing, raises them and so does exposure to wind. Increasing fatness tends to lower the critical temperature of the individual by increasing the insulating layer under the skin. Food intake results in an increase in heat production which has the practical effect of lowering the critical value.

As the environmental temperature is raised above the critical, physical regulation operates without any increase in metabolism until this regulation becomes insufficient to cool the body. At this point a supernormal body temperature ensues, referred to as the *hyperthermal rise*. This in turn results in an increased metabolism. The range of temperature between the critical and the point of hyperthermal rise is referred to as the *range of thermal neutrality*, and it is in this range that basal metabolism studies are made (Sec. 222).

**205. The Measurement of Heat Production.**—By using the same principle that is employed in measuring the heat produced by burning a feed in a bomb calorimeter, the heat of body oxidation processes may be measured by enclosing the animal in a specially constructed chamber called an *animal calorimeter*. Such an apparatus for use with pigs is described by Deighton.<sup>1</sup> Strictly speaking it is the heat given off, and not necessarily the heat produced, which is thus measured though we commonly refer to it as heat production.

**206. Respiration Calorimeter.**—The direct measurement of heat production is commonly carried out in a respiration calorimeter which combines the features of a respiration chamber and a calorimeter. Such an apparatus makes possible an accounting for the income of feed, water, and oxygen and the outgo of the solid, liquid, and gaseous excreta and of the heat production. In the lactating animal, milk outgo can also be accounted for. Shortly after Atwater and his associates perfected their respiration calorimeter for use with man, Armsby<sup>2</sup> built a similar one for

<sup>1</sup> DEIGHTON, THOMAS, A new calorimeter for use with young farm animals, *J. Agr. Sci.*, 16, 376–382, 1926.

<sup>2</sup> Henry Prentiss Armsby (1853–1921), following periods of service at the New Jersey, Connecticut, and Wisconsin Experiment Stations, became



experiments with cows at Pennsylvania State College, introducing certain modifications required for use with animals. This calorimeter is still in active service at the Institute of Animal Nutrition of the College. It is the only one in operation with cattle in the world today. The following brief description of it is taken from a recent bulletin by Braman.<sup>1</sup>

A horizontal cross section of the respiration calorimeter is shown in Fig. 24, and a view of the interior is given in Fig. 25. The three walls, with the air spaces in between, provide the special construction which prevents the chamber from gaining or losing heat. This is accomplished by maintaining the temperature in the air spaces the same as in the chamber by means of water pipes for cooling and resistance coils for heating, both of which are located in the air spaces. The necessity for heating or cooling is determined by a large number of thermocouples in the walls of the inner metal chamber and of the wooden chamber next outside. This control is so delicate that changes as small as  $0.01^{\circ}\text{C}$ . can be detected and immediately corrected for.

The heat produced by the animal is removed as fast as produced, by cold water flowing through a series of pipes suspended from the ceiling of the chamber. The heat thus removed is translated into calories by recording the difference in temperature between the ingoing and outflowing water and the water volume. This heat plus the latent heat of vaporization of moisture condensed on the heat-absorbing apparatus or carried out in the air current gives the heat production.

A steady flow of air is drawn in and out of the chamber by a special pump, and its volume is measured by passing it through a meter. The entrance flue is equipped with both heating and

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director of the newly established Pennsylvania Experiment Station at State College in 1887. In 1907 the Institute of Animal Nutrition was established at this institution with Armsby as director, and here he served until his death, winning lasting fame for himself and his Institute. During his postgraduate study at Leipzig, he became interested in the respiration experiments being carried out by Kuhn and others at Mockern, and this resulted in his construction of a respiration calorimeter for farm animals and in the inauguration of his studies of heat production in cattle. These epoch-making studies, which led to the development of the net-energy system of evaluating feeds, are frequently referred to in this book.

<sup>1</sup> BRAMAN, WINFRED W., The respiration calorimeter, *Pa. Agr. Expt. Sta. Bull.* 302, 1933.

cooling facilities to keep the temperature of the incoming air identical with that of the outgoing, thus avoiding the error which would otherwise be introduced in the measurement of heat production. A continuous sample is drawn from the incoming air for the determination of water and carbon dioxide by absorption and of any methane by combustion. Continuous samples are taken from the outgoing air for the same determinations, and there is also provision for analyzing this air for oxygen. These measurements, together with analyses of the chamber air at the

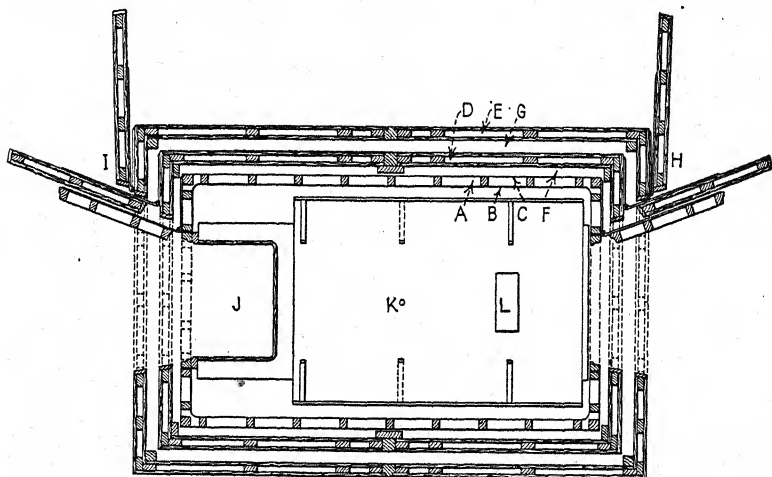


FIG. 24.—Horizontal cross section of the Armsby respiration calorimeter. *A*, double metal wall; *B*, inner wall of copper; *C*, outer wall of zinc; *D* and *E*, double wooden walls; *F* and *G*, air spaces; *H*, entrance doors; *I*, feedbox doors; *J*, feedbox; *K*, opening for urine tube; *L*, opening for feces hopper. (Courtesy of E. B. Forbes, Institute of Animal Nutrition.)

beginning and end of the period, provide the data for gaseous exchange.

It is noted in the diagram that there are openings for the discharge of urine and feces. The one for urine is for use with a steer equipped with a harness holding a tube leading to a urine receptacle under the floor. Similarly, there is special equipment for guiding the feces through *L* into a container underneath. These features provide for the quantitative collection of the excreta. In addition to the arrangement for feeding as indicated by the feedbox, there is also provision for watering. There are many other features, such as devices for recording the movement

of the animal and for accounting for the proportion of the time spent standing and lying down. For further details of the construction and operation, the student is referred to the descriptive bulletin.

A test in the respiration calorimeter is normally preceded by a considerable preliminary period during which the animal receives

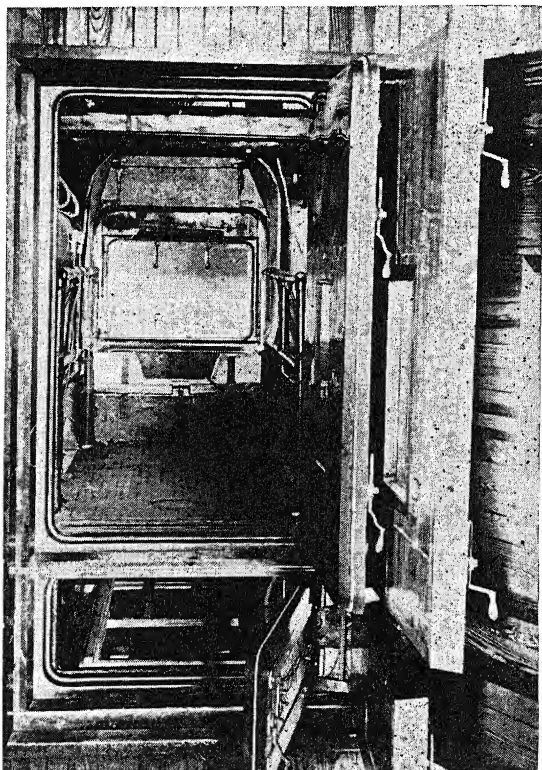


FIG. 25.—Interior of the Armsby respiration calorimeter. (Courtesy of E. B. Forbes, *Institute of Animal Nutrition*.)

the same feed intake as that during the test. A digestion trial is commonly carried out during the preliminary period. Of course, the digestion trial, as well as feeding during the test itself, is omitted when fasting metabolism is being studied. An accounting is made for insensible losses from the animal by weighing it at the beginning and end and by taking into account the weights of

food, water, and excreta. It is evident that the conduct of a test in the respiration calorimeter requires a high degree of skill. The accuracy of the apparatus itself is amazing. The heat and gaseous products of combustion which are obtained by burning a known amount of alcohol in a special lamp in the chamber are generally found to be within less than 1 per cent of the theoretical values.

An illustration of the data obtained in an experiment carried out in a respiration calorimeter is given in Table XIX. It is noted that an accounting for all energy losses left a balance of 608 Cal. as the net gain to the animal from the feed ingested. The striking feature of these data is the large loss of energy as heat, representing approximately 40 per cent of the total intake. The importance of giving attention to heat losses in measuring the usefulness of feeds is thus indicated.

TABLE XIX.—DAILY ENERGY BALANCE OF A STEER<sup>1</sup>

Items	Income, Cal.	Outgo, Cal.
6,988 g. timothy hay.....	27,727	
400 g. linseed meal.....	1,811	
16,619 g. feces.....		14,243
4,357 g. urine.....		1,210
37 g. brushings.....		88
142 g. methane.....		1,896
Heat.....		11,493
Gain by body.....		608
Total.....	29,538	29,538

<sup>1</sup> Data from ARMSBY, HENRY PRENTISS, and J. AUGUST FRIES, The available energy of timothy hay, *U. S. Dept. Agr. Bur. An. Ind. Bull.* 51, 1903.

**207. Net-energy Values.**—In connection with his studies with the respiration calorimeter, Armsby developed his net-energy system of evaluating feeds founded upon a concept not basically different from that underlying Kellner's starch values (Sec. 199). While not agreeing that it was literally "work of digestion" in its entirety, Armsby recognized that the assimilation of a feed resulted in an energy cost to the organism in addition to those losses accounted for in arriving at metabolizable energy, and that

this energy cost could be measured as heat production. He therefore measured the heat production of a feed at a given level of intake, increased the intake, and, by a second measurement of the heat production, obtained by difference the *heat increment* (Sec. 216) corresponding to the amount by which the level of food intake was increased. He then subtracted the heat increment, expressed in terms of a given unit of intake, from the metabolizable energy of the same intake to obtain the net-energy value. In the case of concentrates, it was necessary to add them to a basal roughage ration in measuring their net-energy value with steers. Some of Armsby's values and the data on which they were based are given in Table XX.

TABLE XX.—NET-ENERGY VALUES OF FEEDS FOR RUMINANTS<sup>1</sup>  
(Therms per 100 lb. of Dry Matter)

Feeds	Gross energy	Losses in excreta	Metabolizable energy	Heat increment	Net energy
Timothy hay.....	204.94	120.84	84.10	35.47	46.63
Red clover hay.....	202.40	111.63	90.77	44.13	46.64
Corn stover.....	196.50	107.96	88.54	48.31	40.23
Corn meal.....	201.49	50.58	150.91	58.33	92.58
Hominy feed.....	213.60	53.84	159.76	61.92	97.84
Wheat bran.....	205.57	91.67	113.90	53.59	60.51

<sup>1</sup> Data from ARMSBY, HENRY PRENTISS, and J. AUGUST FRIES, Net energy values for ruminants, *Pa. Agr. Expt. Sta. Bull.* 142, 1916.

Since the direct determination of net-energy values was necessarily slow and expensive, Armsby and Fries developed, from respiration calorimeter experiments, factors for computing metabolizable energy values from digestible organic matter. Given the metabolizable energy for a feed, they subtracted the value for its heat increment either directly determined or estimated from the value for a similar feed. In this way they prepared a table of net-energy values for the common feeds. Later studies by Forbes and Kriss<sup>1</sup> resulted in improved methods of computing these values from Armsby's data and in the publication of a table of revised values.

<sup>1</sup> FORBES, E. B., and MAX KRISS, Revised net-energy values of feeding stuffs for cattle, *J. Agr. Research*, 31, 1083-1099, 1925.

It is evident that both Kellner and Armsby arrived at essentially the same measure of feed values; the former by calculating the productive value as measured by the gain in fat shown by the carbon-nitrogen balance, the latter by directly accounting for all losses and thus arriving at a value which represented energy gained. In fact, Kellner stated his gains in fat in Calories, and he computed the percentage of metabolizable energy thus stored, but he expressed the energy value of his feeds in terms of matter instead of Calories, because he felt that starch values would be more readily understood in practice. Armsby regarded Kellner's figures as real net-energy values, and he made use of them in preparing his own table of values. A critical discussion of the usefulness and limitations of these values is presented later (Chap. XI).

**208. Indirect Calorimetry.**—The measurement of heat production as just described is referred to as *direct calorimetry* in contrast to *indirect calorimetry* in which the heat production is calculated from data as to the chemical metabolism. Such a calculation is possible if the complete chemical metabolism is known since every chemical process is related to a definite transformation of energy. Fortunately, it is not necessary to know all the chemical changes, but only the initial and final states, because the sum of all transformations of chemical energy results in a heat production which is independent of any variations in the intermediary processes (Law of Hess). Heat production can thus be computed either from the data of a nitrogen and carbon balance or from the data of gaseous exchange.

The procedure of the first method, most applicable to animals receiving feed, may be illustrated from the work of Armsby. He used the data of nitrogen-carbon balances to compute heat production in order to compare the values thus obtained with those actually observed in the calorimeter. For example, data obtained in connection with the energy balance listed in Table XIX showed gains of nitrogen and carbon which corresponded to gains of 66.6 g. of protein and 15.2 g. of fat. On the basis that the energy value of each gram of protein stored is 5.7 Cal. and of each gram of fat, 9.5 Cal., he calculated that the total energy thus gained was 524 Cal. The metabolizable energy was calculated from the data in the table by subtracting all outgo except heat from the total income, giving a figure of 12,101 Cal. Subtracting

the figure for energy gained as protein and fat, 524 Cal., from the metabolizable energy left a balance of 11,577 Cal., which represented the energy lost as heat, as thus determined by indirect calorimetry. These calculations both illustrate the method of computing heat production from the nitrogen-carbon balance, and indicate the accuracy of the method, since the value thus obtained by indirect calorimetry closely approximates the directly determined value of 11,493 Cal. as given in Table XIX.

Instead of obtaining the changes in protein and fat in the body as described in the preceding paragraph, these changes can be directly determined by the slaughter method. This procedure, along with a measure of urine and fecal losses, suffices to arrive at the heat production indirectly, in animals where combustible gases do not figure. This method has been used for the rat and is applicable to the pig.

Heat production may be computed from oxygen consumption by use of the average heat equivalent of a liter of oxygen. It may be more accurately calculated from the determination of both the oxygen consumption and carbon dioxide output, which thus gives the respiratory quotient (R.Q.) and thereby makes possible the use of an exact value as the heat equivalent for the oxygen consumed. Knowing the R.Q., the proportion of fat and carbohydrate being burned, and thus the energy being produced per liter of oxygen consumed at that R.Q., can be computed. This calculation has been made for all respiratory quotients between 1.0 and 0.7, and the values are available in tabular form (Zuntz and Shumberg). Thus, if the data of a respiration experiment show that 70 liters of oxygen were consumed and that the R.Q. was 0.9, multiplying this number of liters by 4.924, the calorific equivalent given in the table for a liter of oxygen at this R.Q., gives 344.7 Cal. as the heat production.

**209. Respiration Apparatus for Indirect Calorimetry.**—The respiration calorimeter experiments of Armsby clearly showed the value of heat production studies with farm animals, and they also indicated that the indirect methods previously employed could be highly useful. Studies by indirect calorimetry were thereby stimulated in view of the much lower cost of construction and operation of a respiration apparatus than of a calorimeter. In this country modern apparatus have been built and described by



Ritzman and Benedict,<sup>1</sup> by Mitchell,<sup>2</sup> and by Kleiber.<sup>3</sup> Though each has its own special features as regards construction and operation, basically they do not differ from the respiration calorimeter with the exception of the absence of the calorimetric feature. It is evident that the construction of an airtight chamber is much simpler than of one which must be heat tight as well. For a detailed description of the construction and operation, the student is referred to the publications cited above. These respiration apparatus have made possible the much more rapid accumulation of data regarding the energy metabolism of various farm animals than would have been possible had such studies been limited to the use of the respiration calorimeter alone. It is generally agreed that the data are highly reliable, closely approximating those obtainable in the calorimeter.

**210. Insensible Loss in Weight as a Measure of Heat Production.**—The loss of body weight which was early referred to as a loss due to “insensible perspiration” is equal to the difference between the carbon dioxide, water vapor, and methane emitted from the body and the oxygen intake. It can be computed from data as to change in weight and as to food intake and fecal and urinary outgo, as is illustrated by the experiment with cattle by Kriss.<sup>4</sup> It was early recognized that the percentage of water vaporized from the skin and lungs is remarkably constant under specified conditions. Since the vaporization of water is the principal channel of heat elimination, other than through radiation and conduction, its measurement has been suggested from time to time as an indirect method of obtaining the heat production. This method has recently been studied with steers by Mitchell and Hamilton<sup>5</sup> with the conclusion that the heat produc-

<sup>1</sup> RITZMAN, ERNEST G., and FRANCIS G. BENEDICT, Simplified technique and apparatus for measuring energy requirements of cattle, *N. H. Agr. Expt. Sta. Bull.* 240, 1929.

<sup>2</sup> MITCHELL, H. H., et al., The effect of the amount of feed consumed by cattle on the utilization of its energy content, *J. Agr. Research*, 45, 163–191, 1932.

<sup>3</sup> KLEIBER, MAX, The California apparatus for respiration trials with large animals, *Hilgardia*, 9, 1–70, 1935.

<sup>4</sup> KRISS, MAX, The insensible loss in body weight of cattle, *J. Agr. Research*, 40, 271–281, 1930.

<sup>5</sup> MITCHELL, H. H., and T. S. HAMILTON, The estimation of the heat production of cattle from the insensible loss of body weight, *J. Agr. Research*, 52, 837–852, 1936.



tion can be estimated in this way with small error from a careful determination of the insensible loss over a two-week period under standardized conditions. The procedure involves a preliminary estimate for each animal of the fraction of the heat lost in the vaporization of water under the same conditions which are to be maintained in the subsequent experiment. It also requires a fairly accurate estimate of the respiratory quotients to be expected on the experimental rations.

Despite the fact that various methods of indirect calorimetry have proven useful and that the methods are much more practicable from the cost standpoint, it is recognized that a distinct place still remains for studies by the direct procedure. Such studies are obviously essential as a check on the accuracy of indirect determinations. There are questions as to the applicability of the indirect method under extreme conditions such as are represented by respiratory quotients greater than 1.0 or less than 0.707 which require comparative study by the use of both methods.

**211. Usefulness of Energy Studies.**—In these days when so much emphasis is being placed upon the more alluring studies of vitamins and minerals, it should not be forgotten that energy metabolism is concerned in every body function and that its study is making equally important, though less spectacular, contributions to our knowledge of the physiology of animal nutrition. Energy studies are concerned with the determination of relative food values, with the influence of various environmental factors, methods of feeding, and combinations of nutrients upon food utilization, and with the efficiency of various animals as converters of food energy. The methods and results of energy studies in these various fields will become evident in later discussions.

**212. Advantages and Limitations of the Balance Method.**—The balance method provides exact information as to metabolic processes and as to the effect of a specific nutrient or ration which cannot be obtained by the more general measures of weight and size, and such studies can be made repeatedly without sacrificing the animal as is required by the slaughter method. It is necessarily an expensive and laborious procedure which must be limited to a few animals and for short periods. The animals must be kept in confinement and subjected to other unnatural conditions.

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## CHAPTER XI

### MEASURES OF TOTAL NUTRITIVE ENERGY

The expression total nutritive energy is used to denote the value of food for its largest function, *viz.*, to furnish energy for body processes and to form the nonnitrogenous, organic matter of tissues and secretions, functions in which all organic nutrients can take part. Total digestible nutrients, starch values, metabolizable energy, physiological fuel values, and net energy, the determinations of which were discussed in the two preceding chapters, are all measures of total nutritive energy. These measures are used to evaluate feeds and rations in experimental work and in practical feeding operations, and they form the basis of various feeding standards which serve as guides in computing rations for various purposes. Since these measures differ as regards the actual feeding value represented, it is desirable to understand clearly the exact significance of each and to know something of their advantages and limitations for practical use.

**213. Total Digestible Nutrients.**—Previous discussion has indicated that, in order to arrive at the actual useful portion of a ration, we must deduct losses in the feces, urine, combustible gases, and heat production. It is obvious that the determination of digestibility is only one step in this direction and thus that digestible nutrients must not be considered as the final measure of useful energy because they are subject to several losses in the course of metabolism. The fecal loss, however, is a large one, exceeding the sum of all the others, at least in the case of roughages. It is a loss which may vary widely among feeds which are alike as regards content of gross energy or total nutrients. Thus it is evident that the determination of digestibility is a highly useful measure, not only because it eliminates an important loss, but also because, by the elimination of a highly variable loss, it places the feeds upon a much more nearly comparable basis as regards their actual physiological value.

Of all the measures of total-nutritive-energy value, digestible nutrients are the easiest to determine. This largely accounts for

the fact that digestion coefficients are available for all of the common feeds. While it is evident that some of them need redetermination, we have far more extensive and more reliable data regarding digestible nutrients than for any other measure. This is an important consideration from the standpoint of their usefulness in practice.

In the previous discussion (Sec. 188) it has been brought out that many digestion coefficients must be determined by difference rather than directly and that all of them are subject to variability according to the combination of feeds used in the ration, the plane of intake, and other factors. That they are not constants must be recognized, but it should also be borne in mind that these various limitations apply with equal or greater force to all of the measures of total nutritive energy.

**214. Metabolizable Energy.**—In taking account of the losses in the urine and combustible gases as well as those in the feces, metabolizable energy represents a step beyond digestible nutrients as a measure of nutritive value. It falls short of being the final measure in that a portion of it is lost as heat not useful to the body, but it is clearly a better measure than digestible nutrients. Over against this must be balanced the fact that the determination of metabolizable energy requires the use of a respiration chamber for those species for which combustible gases are an important loss, involving a much more costly and laborious procedure than is needed for the determination of digestibility alone. It may be questioned whether the additional information gained is sufficiently important from the standpoint of use in feeding practice to justify the large outlay involved. It is possible, however, to compute the output of combustible gases from carbohydrate intake and even to compute metabolizable energy from digestible nutrients as Armsby did as a basis for arriving at many of his net-energy values.

At the present time, actually determined values for metabolizable energy are available for only a few feeds, and there seems to be no large current interest among investigators in this measure of energy value. It is evident that any variable factors which influence digestibility also affect metabolizable energy. Mitchell and coworkers<sup>1</sup> reported that the metabolizable energy per kilo-

<sup>1</sup> MITCHELL, H. H., and coworkers, The effect of the amount of feed consumed by cattle on the utilization of its energy content, *J. Agr. Research*, 45, 163-191, 1932.

gram dry matter of a ration for steers increased progressively from 2661 Cal. to 3094 Cal. as the level of intake was reduced by stages from full feed to one-fifth of this intake. Digestibility and the percentage of metabolizable energy utilized also increased.

**215. Physiological Fuel Values.**—The calorific values obtained by the use of the physiological fuel values (Sec. 203) applied to data on the analysis of foods provide a measure similar in principle to metabolizable energy. They are used in connection with rations for Carnivora as well as man, and they are applicable for computing the calorific value of diets fed in rat experiments in which the ingredients are similar in nature to human foods. It must be remembered that they are based upon average digestion coefficients obtained with the mixed human diet which is low in crude fiber. As the fiber content increases, digestibility falls below the percentages used in calculating the physiological fuel values of the nutrients, and these values become too high accordingly. This is the situation that exists for most human, vegetarian diets, particularly those based upon unrefined foods, and it constitutes the reason why the values are not applicable in the case of Herbivora. None of the rations of our farm Herbivora are as completely digested as is indicated by the percentages used in computing physiological fuel values.

### NET ENERGY

The net-energy system, whether expressed in terms of Kellner's starch values or of Armsby's net-energy values, conceives of the measurement of that portion of the feed which is actually useful to the body. By taking account of all losses in metabolism, net energy, according to this concept, is the actual and complete total-nutritive-energy value of a feed or ration for a given body function. Theoretically, 1 Therm of feed energy corresponds to 1 Therm actually utilized. On this basis it certainly represents a more nearly exact measure than any of the others which fail to account for one or more of the losses always involved. The distinguishing feature of the net-energy system is an accounting for the loss as heat increment of that portion of the metabolizable energy which fails of conversion into body substance or product, a loss which varies significantly from one feed or ration to another.

**216. Heat Increment.**—The measurement of heat increment, whether carried out in the respiration calorimeter or determined

indirectly by one of the methods previously described (Sec. 208), is a procedure which gives highly reliable results for a given set of conditions. The instrumental error involved in its determination is only 1 or 2 per cent. The heat increment of a given feed, however, is subject to several factors of variability according to the conditions under which it is measured, and this fact has an important bearing on the usefulness of the net-energy system as a measure of total nutritive energy.

The heat increment of a feed or ration can be measured between any two planes of intake, but determinations at submaintenance levels have a different significance from those determined above maintenance. It is now recognized that the usual purpose of the determination is best served by making maintenance the lower plane. The determination is made in a thermal environment at or above the critical temperature and below the point of hyperthermal rise and, thus, above the point below which the oxidation of body substance becomes necessary to keep up body temperature. It is recognized that a heat loss thus measured has a tissue-saving value for an animal in an environment below its critical temperature and thus that the net-energy system may undervalue certain rations for animals maintained under cold conditions in practice. It is now agreed that the additional work of digestion is a minor factor in heat increment. Differences in muscular activity or tonus<sup>6</sup> play some role, but such differences are largely corrected for in making the measurements. For example, a record is kept of the time the animal spends standing and lying down, and the results are corrected to a standard day of 12 hr. of each. The primary cause of differences in heat production lies in variations in the *specific dynamic effect* which is defined in an exact sense as the heat increment of a specific kind of nutriment.

It has long been recognized that the ingestion of pure, readily absorbable nutrients results in an increased heat production which is the most marked in the case of protein. No simple explanation of the cause of this heat increment finds general acceptance, and it is probable that several factors are involved. Voit considered it to result from a direct stimulus to oxidative metabolism of the absorbed nutrients within the cells of the tissues, a stimulus which was proportional to the concentrations attained. According to this theory, therefore, heat increment is

caused by a plethora of nutrients in the tissues. Rubner, on the other hand, believed that the heating effect of ingested food was the result of intermediary oxidations and reactions causing the liberation of energy which was only in part available for physiological work, the balance being dissipated as extra heat. Both of these theories have their adherents, and they provide different explanations of the variations in heat increment which are not only puzzling, but also upsetting, in so far as the usefulness of net-energy values is concerned.

Whatever may be the cause of specific dynamic effect, it is agreed that the summation of such values for the individual nutrients in a feed does not necessarily represent the heat increment of the feed itself, and further that the heat increment of a ration is not necessarily the sum of the values of its feed components. From this it follows that the net-energy values of individual feeds are not strictly additive. The value of the mixture may be greater or less than calculated, depending upon how the heat increments of the individual nutrients and feeds are modified by the combination. For example, Forbes and coworkers<sup>1</sup> found that, considering the value for a given basal ration as constant, the addition of corn meal to different basal rations resulted in variable figures for the heat increment and thus for the calculated net-energy value of the meal according to the ration with which it was fed. The experiment showed, however, that net energy is not the only measure subject to this variability, for the digestibility and metabolizable energy of the corn differed with the different basal rations.

Another limiting factor in the usefulness of net-energy values is that the heat increment per unit of food increases with level of intake, but not in any simple relationship which allows this factor of variation to be accounted for accurately by calculation. The effect is undoubtedly due to the interplay of several factors which are explainable in various ways according to the theory of specific dynamic action adhered to. The practical result is that for a given food or combination there is a different net-energy value at each point of observation as the plane of nutrition changes.

<sup>1</sup> FORBES, E. B., WINFRED W. BRAMAN, MAX KRISS, R. W. SWIFT, ALEX. BLACK, DONALD E. FREAR, O. J. KAHLENBERG, F. J. McCLURE and LEROY VORIS, The associative effects of feeds in relation to the utilization of feed energy, *J. Agr. Research*, 46, 753-770, 1933.

**217. Net-energy Value and Body Function.**—Since the heat increment varies with plane of intake, it is to be expected that a given feed will have a different energy value when fed below the maintenance level than above and a different value for growth or fattening than for maintenance alone. It is of greater significance that, at the same level of intake, a given feed has a different net-energy value according to the productive function being supported. For example, the workers at the Pennsylvania Institute of Animal Nutrition have found that 1 Therm of metabolizable energy is equivalent to 0.693 Therm of milk energy but only 0.575 Therm for fattening. It is thus clear that the proportion of the metabolizable energy lost as heat differs according to the body function for which it is being used, and this is understandable in view of the differences in the metabolic processes involved. From the practical standpoint, this means that there can be no one set of net-energy values for our feeds but that there are different values for different purposes, in contrast to digestible nutrients which remain invariable in this respect since they take no account of body processes beyond the digestion stage. This variability of net-energy values according to body function adds to the difficulty of providing the necessary data for making use of the net-energy system in the various operations of feeding practice, but it does not affect the soundness of the system itself.

**218. Net Energy and Balance of Nutrients.**—In general terms a balanced ration is one which supplies all the nutrients needed for the optimum nutrition of the body function in question in adequate amounts and in proper relationships to each other. It is a ration which is qualitatively and quantitatively complete. Ideally it should support to the maximum the function for which it is balanced, but there should be no excess of any nutrient or of the ration as a whole large enough to interfere with physiological efficiency. This concept of a balanced ration does not necessarily imply that all of the essential nutrients must be present in certain fixed proportions. Rather there may be a certain range between the minimum optimum level and the upper level of efficiency within which the proportion of a given nutrient may fluctuate without affecting the balance.

There is evidence that the more nearly balanced the ration the smaller the proportion of it which is dissipated as heat and the



greater, therefore, is its net-energy value. A deficiency of one nutrient impairs the efficiency of all others. It has been shown, for example, by Swift and associates,<sup>1</sup> at the Pennsylvania Institute of Animal Nutrition, in experiments with rats that the addition of 0.24 per cent of cystine to a ration unbalanced with respect to this amino acid resulted in a storage of 10.8 per cent more energy as well as a greater growth in weight and a storage of 24.4 per cent more nitrogen. An additional storage of 40.4 Cal. in the course of 14 weeks was accomplished by 29 Cal. less loss as heat, 8.6 Cal. less loss in the feces, and 2.8 less loss in urine.

Studies at the Institute of Animal Nutrition, as well as at the University of Illinois, have also shown that the percentage of total protein has a very definite effect on the heat losses. In the Illinois experiments<sup>2</sup> it was found with rats that, as the protein level was increased from 4 per cent to 18 per cent, there was a progressive decrease in the heat produced. For example, a ration containing 8 per cent of protein had a heating effect averaging 443 small cal. per gram, compared to only 80 small cal. for the ration containing 18 per cent. These decreased heat losses with increasing protein level had the practical effect of increasing the net-energy value. This finding is in direct conflict with the prevailing view that increasing the protein content of the diet increases its heating effect because protein has a larger specific dynamic effect than do other nutrients. It illustrates the fact that these effects of a specific kind of nutriment are not additive and that the heat losses of the ration as a whole depend upon the extent to which it is balanced in terms of all the factors which contribute to its nutritive value. Further evidence for this viewpoint comes from the most recent report from the Institute of Animal Nutrition showing that sodium deficiency increases heat losses.<sup>3</sup>

<sup>1</sup> SWIFT, R. W., O. J. KAHLENBERG, LEROY VORIS, and E. B. FORBES, The utilization of energy producing nutriment and protein as affected by individual nutrient deficiencies. I. The effects of cystine deficiency, *J. Nutrition*, **8**, 197-219, 1934.

<sup>2</sup> MITCHELL, H. H., and T. S. HAMILTON, The balancing of rations with respect to protein, *Proc. Am. Soc. Animal Production*, 1935, pp. 241-252.

<sup>3</sup> KAHLENBERG, ORME J., ALEX BLACK, and E. B. FORBES, The utilization of energy producing nutriment and protein as affected by sodium deficiency, *J. Nutrition*, **13**, 97-108, 1937.

From results such as the preceding, the conclusion has been reached that each improvement in nutritive balance results in a better utilization of metabolizable energy by economizing heat losses and thus increases the net-energy value. Whatever may be the true explanation of specific dynamic effect, the efficiency of the utilization of metabolizable nutrients must depend upon how closely their proportions correspond to those which can be effectively used for the body processes in question, and it seems logical to expect that the poorer the balance the greater the "leftovers" which would be especially subject to dissipation as heat. In fact from one point of view, one may think of heat production as a defense mechanism by means of which excessive concentrations of nutrients are removed. Thus heat increment increases with plane of intake because the concentration of nutrients is augmented owing to a failure of the rate of utilization to keep pace with intake, and it is further increased when there is a lack of balance, because "leftovers" tend to increase the concentration still further. This is most easily visualized according to the Voit theory which conceives of heat production as being due primarily to a plethora of nutrients in the cell, the magnitude of which is governed by both the supply and the rate of utilization. It is recognized that heat production is at a minimum when just the amounts of protein, fat, and carbohydrate required to meet body needs are given in frequent feedings.

Since individual feeds are seldom if ever balanced rations, it is evident that the summation of the net-energy values of the feeds making up a ration is not an accurate measure of the energy value of the ration as a whole and that the values for certain individual feeds may be highly misleading as to their effects as constituents of completely balanced rations. Herein lies another practical limitation of the net-energy system.

**219. Inconstancy of Net-energy Values.**—A consideration of these various factors which influence the heat increment has led to the conviction that net-energy values of individual feeds cannot be considered in any sense as constants, that they are so variable as to be of very limited usefulness for arriving at the energy value of rations representing different combinations and fed at different levels. It has come to be recognized that net-energy values have their most nearly exact and characteristic meaning when they are determined for the ration as a whole which is completely balanced

for the body function in question. The logic of this viewpoint is clear, but its implications in terms of the usefulness of the net-energy system must be recognized. Our knowledge is still too imperfect to decide what is a completely balanced ration in terms of all the qualities which are implied in its definition. Further, the determination of the net-energy values of all of the balanced rations which find use in practice is clearly impossible. To what extent calculations based upon certain typical combinations could be made use of is a question that can be answered only by further study of the net-energy system.

At this point it is worth bearing in mind that none of the other methods of measuring total nutritive energy is free from the variables which affect the net-energy system. It has been noted, for example, that the digestibility of an individual feed is not a constant but may vary according to the plane of intake and that the digestibility of a ration as a whole cannot be exactly predicted from the coefficients of its constituents. The factor of associative digestibility enters, and so does nutritive balance, at least in so far as the nutritive ratio is concerned. Metabolizable energy is subject to the same variable factors.

From a strict point of view, therefore, it must be considered that no measure of nutritive value is accurate when applied to an individual feed because the latter is not a balanced ration and cannot be fed at a practical production level and that any measure is subject to variability according to the combination in which the feed is used. But in practice we must deal with individual feeds and some measure of value for them is essential. Certainly all of the measures discussed in this chapter, even though not constants, provide information which make them better measures than a mere statement of chemical composition. Further, it must be borne in mind that even an exact measure of the nutritive value of a completely balanced ration is not a constant for different animals, or even for the same animal under varying conditions. There is a rather large factor of biological variability which cannot be eliminated. The imperfections of present measures should be appreciated, and an endeavor made to improve them, but, in the meantime, animals must be fed. Therefore, it should be recognized also that a method may be distinctly useful, although it may not be perfect, particularly in a field in which perfection is an unattainable ideal. Such is the

situation with respect to measures of total nutritive-energy value. The practical problem is to select the most useful measure in terms of present developments.

Net-energy values are subject to more and to larger variables than are other measures because they take into account a further loss, the heat increment, a loss which is particularly susceptible to variability. Thus while no measure can be considered a constant, net energy, which is clearly the most nearly exact measure of useful nutritive energy for a stated feed or ration, condition, and function, becomes, by its very exactness for specified conditions, the more difficult to apply as a workable measure to the varied conditions of practice. The many variable factors which govern the net-energy values of individual feeds have caused authorities working in this field to consider that further determinations of these individual values are not worth while. The values now available, which it is agreed require restudy and revision, are mostly computed ones based primarily upon body increase, and their application to milk production is especially questionable. Data obtained on the preferred basis of balanced rations are too scanty for any practical use. These various considerations lead to the conclusion that the net-energy system does not at present provide a suitable measure of total nutritive energy for practice, though it remains a promising field for further study.

In the meantime the values for digestible nutrients, which are available for all of our commonly used feeds, must be considered the most practical measure, both for comparing feeds and as a basis of feeding standards. That the values for individual feeds are not constants and that they do not take account of all losses must be recognized, but these limitations in their practical use can be lessened by bearing in mind how digestibility is influenced by various factors and by taking into account the available knowledge as to the losses to which digested nutrients are subject in the course of their utilization.

#### OTHER MEASURES OF TOTAL NUTRITIVE ENERGY

Various other methods of measuring total nutritive energy are in use and they have resulted in values which have been made the basis of feeding standards. *Møllgaard's production equivalent system*, which finds use in Denmark, is based upon energy-balance studies with milch cows, and it combines some features of both

Kellner's and Armsby's methods. The production unit is the net energy for fattening which will produce 1000 Cal. of milk energy, viz., 837 Cal. in a ration which is properly balanced with respect to protein.

Hansson's *food-unit system* is based upon results from practical feeding experiments, with 1 kg. of barley as the standard unit. Recently it has been expressed in accordance with the net-energy concept by computations making use of Kellner's starch values. As so computed, one food unit is equivalent to 1.65 Therms net energy for fattening or 2.1 Therms milk energy. These values, as do also those of Møllgaard, recognize the previously mentioned greater net-energy value for milk production than for fattening (Sec. 217).

*Fraps' energy-production coefficients*<sup>1</sup> are values derived from Kellner's work which are applied with certain corrections to the various nutrients in feeds to compute their production energy. The values thus obtained are modified for certain feeds in accordance with the results of feeding trials when the latter suggest the computed values to be in error.

#### NUTRITIVE-ENERGY VALUES FOR FEEDING STANDARDS

Feeding standards are tables showing the amounts of the nutrients which should be provided in the rations of different species for different purposes, such as growth, fattening, and lactation. Commonly these standards deal only with the requirements for protein and total nutritive energy. The protein requirement is generally stated on the basis of the digestible nutrient but the energy requirement is expressed in a variety of ways, making use of the various measures described in this chapter.

**220. Bases of Feeding Standards.**—In 1810, many years before the nature of the organic nutrients in foods was appreciated Thær developed his "hay values" as measures of relative nutritive value. His basic hay value consisted of the sum of the ingredients extractable with water, alcohol, dilute acid, and dilute alkali. Similar determinations made on other feeds were referred to this basic value as the standard. Following the recognition of protein,

<sup>1</sup> FRAPS, G. S., Productive energy of feeds calculated from feeding experiments with sheep, *Texas Agr. Expt. Sta. Bull.* 436, 1931. (See also later papers by this author.)

fat, and carbohydrate as the essential organic nutrients, Grouven made use of analyses for these nutrients to formulate in 1859 the first feeding standard for farm animals.

In 1864 Wolf devised a standard based on digestible nutrients which was derived from results of various feeding trials. His standards were republished annually without fundamental change until modified by Lehman in 1897. In 1914 an important advance in the accuracy of the standard for dairy cows was made, as a result of many years' study by Haecker<sup>1</sup> of the University of Minnesota, showing that the nutritive requirements varied not only with the quantity of milk produced but also with its quality, especially its fat content. In the following words Haecker set forth a principle which should be recognized in all studies of nutritive requirements:

In order to determine the actual net nutrients required to produce a given animal product, the composition of the product should be known, as well as the composition and the available nutrients in food which is to be fed for its production, so that the nutrients in the ration might be provided in the proportions needed by the animal. Before a builder bids on a contract, he determines the quantity needed of each of the materials that are to appear in the structure. Without such specifications he would not know how much of each of the different materials would have to be provided.

Since the time of Haecker several different standards, based upon digestible nutrients have been proposed for farm animals by various American workers. Such is the basis of the Morrison standards which are at present widely used. Both Kellner and Armsby devised standards utilizing the net-energy system and there are also standards by Hansson and by Møllgaard, based on their measures of total nutritive energy. The features of these various standards have been excellently set forth by Kriss.<sup>2</sup> The standards used in human nutrition make use of physiological fuel values.

**221. Usefulness of Feeding Standards.**—Feeding standards are not exact statements of nutritive requirements. Designed to

<sup>1</sup> HAECKER, T. L., Investigations in milk-production, *Minn. Agr. Expt. Sta. Bull.* 140, 1914.

<sup>2</sup> KRISS, MAX, A comparison of feeding standards for dairy cows, with special reference to energy requirements, *J. Nutrition*, 4, 141-161, 1931.

be guides for practice, as well as a basis for teaching, they must allow a factor of safety for individual variability, and their values must be higher than the minimum requirements under optimum conditions. They are not complete statements of nutritive requirements because they commonly deal only with protein and total nutritive energy. The needs for mineral and vitamins and the question of protein quality must also be taken into account. A standard which attempted to cover all of these factors would be a highly complicated one, and, even then, it would not be a complete guide to feeding because other considerations, such as palatability and physical nature of the rations, would still require attention. The fact that feeding standards do not take account of all the factors which comprise a complete and satisfactory ration does not alter their basic usefulness in practice and teaching and even in research.

The status and significance of studies of energy metabolism and requirements and their application to feeding practice, with particular reference to the net-energy system, are set forth by the foremost workers in the field in a recent report of a conference held under the auspices of the National Research Council.<sup>1</sup>

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<sup>1</sup> Report of the Conference on Energy Metabolism, held at State College, Pa., June 14-15, 1935, under the auspices of The Committee on Animal Nutrition of the National Research Council, Washington, D. C.

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## CHAPTER XII

### THE FASTING CATABOLISM. MAINTENANCE

Whether an animal is being fed for growth, fattening, milk secretion, or other productive function, a substantial part of its food is used for supporting body processes which must go on whether or not any new tissue or product is being formed. This demand for food is referred to as the maintenance requirement, since it comprises the amount needed to keep intact the tissues of an animal which is neither growing, working, nor yielding any product. If this need is not met, tissue breakdown occurs, which is commonly revealed by a loss in weight and which leads to various undesirable consequences. For a considerable part of the human population, the maintenance requirement comprises the principal need for food. While this is much less true for farm animals because they are always fed for productive purposes, maintenance is an important "overhead" of the livestock business. The income is governed by the ability of the animal to consume and utilize feed in addition to its maintenance requirement. Thus the knowledge of this requirement, which the student must have in order to understand the principles underlying nutrition, has a direct practical interest. The starting point of this knowledge is the fasting catabolism.

#### THE FASTING CATABOLISM

The animal receiving no food, doing no external work, and yielding no product is nevertheless carrying on a variety of internal processes which are essential to life. These processes include respiration, circulation, maintenance of muscular tonus, manufacture of internal secretions, and several others. In the absence of food, the nutrients required to support these activities must come from the breakdown of body tissue itself. This destruction of body tissue is referred to as the fasting catabolism, and it can be measured in terms of the waste products eliminated

through the various paths of excretion. Most of the breakdown which occurs is in response to the demand of the fasting organism for energy for its vital processes.

**222. Energy Catabolism of Fasting.**—The energy consumed in the fasting animal appears as heat and thus can be measured in the respiration calorimeter, or it can be obtained by one of the methods of indirect calorimetry. Its measurement provides a useful basis of reference for other phases of the energy metabolism. In order that the fasting catabolism may be measured at its minimum value, it is necessary that all influences tending to increase heat production above the minimum expenditure compatible with the maintenance of life be eliminated in so far as possible. Such a minimum value is called *basal metabolism*. It has its most exact meaning in the case of man, because it is with this species that the conditions which are essential for a true minimum value can most nearly be attained. The conditions for its measurement in man are commonly specified as follows:

1. Good nutritive condition
2. Environmental temperature of approximately 25°C.
3. Relaxation on bed prior to and during measurement
4. Postabsorptive state

A good nutritive condition implies that the previous diet of the subject has been adequate, especially as regards energy and protein. A poor state of nutrition tends to decrease the heat production during fasting. The temperature of 25°C. is specified as one which is above the critical, assuring that no tissue breakdown is occurring to keep up the temperature of the body, and as one below the point of hyperthermal rise where the onset of febrile conditions increases heat production. Both of these first two conditions are entirely realizable in the case of animals. The minimum muscular activity assured by the third condition, however, is obviously much less subject to control, particularly in farm animals. In addition to various miscellaneous movements, the animal may be expected to spend a variable portion of the experimental period standing and lying down. The magnitude of the influence of voluntary muscular activity is illustrated by the observations that the basal metabolism of different species and individuals is 10 to 15 per cent greater when they are standing than while they are lying down. In making the measurement, therefore, the heat production is calculated separately for the

periods of standing and lying and then computed to a standard day of 12 hr. of each.

The fourth condition implies a state of fasting in which a long enough time has elapsed since the ingestion of food to make certain that the heat increment due to its digestion and assimilation has been dissipated. Such a condition is readily obtainable in animals with simple stomachs, but not in the case of the ruminant. In this species the anatomy and physiology of the digestive tract result in a prolonged retention of food in the rumen and a correspondingly slow passage through the tract and into the blood stream. The achievement of a truly postabsorptive state cannot be obtained except after such a prolonged period of fasting as may result in other disturbing factors which alter the normal catabolic processes. Thus the measurement of basal metabolism in the ruminant cannot have the exact significance that it does in man.

In the ruminant a minimum value for the methane excretion is one criterion of the establishment of the postabsorptive state. Another criterion of the attainment of a basal condition is a respiratory quotient which indicates that little or no carbohydrate is being burned, a condition that is generally reached after two or three days of fast. On the latter basis the heat production of the next experimental period following the attainment of a metabolism which is characterized by the nonprotein, respiratory quotient of fat (0.707) is frequently referred to as the basal metabolism. Some workers determine what is called a *standard metabolism*, which is a value obtained under specified conditions as to time after the last feeding, and such values are referred to as basal metabolism. While it is preferable from a strict point of view to refer to any value determined on a ruminant as a fasting catabolism rather than a basal metabolism, the usefulness of the determination of such values cannot be questioned. The conditions under which they are obtained should, however, be accurately defined.

Determinations of fasting catabolism for a given species provide a basis for studying the factors which affect this function and for comparing the metabolism in different species. They also provide a base line for measuring the effect of any superimposed factor such as muscular work, digestion, and other body activities.

**223. Units of Reference in Fasting Catabolism.**—Heat production is obviously related to body size. In making use of determined values it is necessary to have some unit of reference. Rubner developed the concept, commonly referred to as the surface-area law, that the heat given off by all warm-blooded animals is directly proportional to their body surface and that, expressed on this basis, heat production is a constant for all species. Thus it became customary to express fasting catabolism in terms of surface rather than of weight, for example, as Calories per square meter per hour. In view of the difficulties and uncertainties involved in measuring surface area, formulas were devised for computing it from weight, recognizing that surface was proportional to some fractional power of weight. Thus most of the values were really based on weight, though expressed in relation to surface.

It is now recognized that the surface-area theory rests primarily on an empirical basis and that it does not have so general an application as previously thought. While the concept has been and still remains very useful, it is agreed that the various methods of measuring or estimating surface area give such variable results that a statement of heat production per unit of surface has a very limited meaning except in terms of the specific method used in obtaining the surface measure. The body surface is not a constant but varies with the position of the body. The fact that the skin is elastic causes its measurement to vary with conditions, whether measured on the live animal or after removal.

In view of these considerations many investigators are returning to the use of weight as the direct unit of reference. Since surface area increases with a fractional power of weight, the metabolic rate can be referred directly to the latter. Different investigators have given figures for this power varying from 0.66 to 0.75. On the basis of an analysis of a very large number of basal metabolism data of mature animals of different species, ranging in weight from 0.02 to 4000 kg. (mice to elephants) Brody and associates<sup>1</sup> suggested the power, 0.73. This value was recommended by the Conference on Energy Metabolism of the Com-

<sup>1</sup> BRODY, SAMUEL, ROBERT C. PROCTER, and URAL S. ASHWORTH, Growth and Development. XXXIV. Basal metabolism, endogenous nitrogen, creatinine and neutral sulphur excretions as functions of body weight, *Mo. Agr. Expt. Sta. Research Bull.* 220, 1934.

mittee on Animal Nutrition.<sup>1</sup> In the case of the human species, however, it remains the general practice to express heat production in terms of surface, calculating the latter from a formula which takes account of both weight and height. Since heat production varies as a fractional power of weight, it follows that it decreases per unit of weight during growth, for the smaller animal has relatively more surface.

**224. Lability of Fasting Metabolism.**—While a properly determined value for basal metabolism is conceived as being a constant, it must be recognized that this is not true in the absolute sense. Differences in the degree of muscle tonus may exist in animals which appear entirely relaxed. The minimum influence of this tonus becomes evident during sleep. Certain internal secretions, notably that of the thyroid gland, augment heat production by increasing the heart rate, the respiration, and, probably, by affecting body oxidations in other ways. Thus variations in the activity of the thyroid influence basal metabolism accordingly. Any marked abnormality of this gland is recognized as pathological, and an altered basal metabolism is expected. The fact that this and certain other pathological conditions are accompanied by characteristic changes in the metabolic rate make the determination of the basal metabolism an important diagnostic agent in the case of various diseases in man. The lowering of basal metabolism which results from castration is due presumably to alterations in the endocrine relationships. This lowered basal-energy requirement is reflected in the cheaper feed costs of gains in castrated farm animals. Basal metabolism decreases with age.

Some rather large deviations in the fasting catabolism of ruminants, not adequately explainable by the recognized factors of variability, have recently been noted. In the case of sheep on pasture in Australia, marked variations according to the season of the year have been reported for values obtained after 48 hr. of fast. Perhaps a true seasonal factor was involved, or perhaps differences in previous feeding were not eliminated by the short period of fast. Benedict and Ritzman,<sup>2</sup> however, have reported

<sup>1</sup> Report of the Conference on Energy Metabolism, held at State College, Pa., June 14-15, 1935, under the auspices of the Committee on Animal Nutrition of the National Research Council, Washington, D. C.

<sup>2</sup> BENEDICT, F. G., and E. G. RITZMAN, Lability of the basal metabolism of the dairy cow, *Proc. Nat. Acad. Sci.*, **21**, 304-308, 1935.

from studies with dairy cows that the fasting metabolism may vary for a given animal as much as 30 to 80 per cent within a period of two months, without any marked change in body weight. Their studies were made under conditions which closely approximate the basal condition. These investigators mention the observation of less marked variations in sheep and horses. Large variations in the fasting catabolism may be exceptional rather than usual, but an important field for further study is here presented, because the possibility of extensive variations obviously must limit the general application of determined values.

As illustrative of the fasting metabolism values for animals reported in the literature, the following, expressed in Calories per square meter per 24 hr., may be cited: horse (lying), 1719; cow (standard day), 1409; sheep (lying), 1163, pullets, 805; dog, 1150. These figures are averages of values representing in most cases a rather wide range for different individuals.

**225. Endogenous Nitrogen Metabolism.**—There is a minimum essential nitrogen catabolism incident to the maintenance of the vital processes of the body, even as is the case for energy. This catabolism is measured as the minimum endogenous nitrogen (Sec. 97) on a nitrogen-free, energy-adequate diet. Upon the inauguration of a nitrogen-free diet the urinary nitrogen decreases gradually. After the attainment of a postabsorptive state as regards protein, there remains "deposit protein" (Sec. 95) to be eliminated, at least in part, before the minimum endogenous value is reached. Thus the higher the level of previous nutrition, the larger the store of "deposit protein" and the longer the time to reach the minimum level. It may be reached in a week with a rat previously on a low-protein diet, whereas on a high-protein diet four weeks or longer may be required. The minimum endogenous nitrogen is the maximum reduction of nitrogen waste of which the body is capable. It may represent a balance between destruction and synthesis—the net catabolism.

In order to arrive at a true value for endogenous nitrogen, it is essential that the animal be receiving a diet adequate in energy, because otherwise the output of urinary nitrogen may include some from body protein which has been broken down to furnish energy and thus be in excess of the value representative of the minimum essential nitrogen catabolism. Muscular activity has no appreciable influence on endogenous nitrogen so long as the

energy intake is sufficient to cover it, for such activity has little, if any, influence on protein catabolism (Sec. 346).

While the measurement of the minimum endogenous nitrogen metabolism is simple in theory, it is difficult in practice to obtain reliable and significant values, particularly with certain species. Not only is a variable and, frequently, a long time required to arrive at what may, by reason of its constancy, be considered a minimum value; but it is difficult, if not impossible, to get most animals to eat a sufficient amount of a nitrogen-free diet for any extended period. Any marked failure of adequate consumption destroys the significance of the results. To obviate this difficulty some investigators feed a small amount of protein of high biological value, which is considered not to affect the urinary output.

#### 226. Relation of Endogenous Nitrogen to Energy Catabolism.

Like basal metabolism, endogenous nitrogen metabolism is a function of body size. Since this is true and since both represent the minimum catabolism essential to life, one would expect a relationship between them. That a relationship actually exists was first proved by Terroine and Sorg-Matter.<sup>1</sup> In this and later studies from Terroine's laboratory, it was found that the ratio of endogenous nitrogen to basal metabolism was nearly the same for mice, rats, pigeons, chickens, rabbits, and pigs and that varying the environmental temperature from 0° to 30°C. did not change it. This work resulted in the conclusion that the *law of constant relationship of minimum nitrogen and energy output* holds for all warm-blooded animals irrespective of body weight or age.

In most of his studies, Terroine included the metabolic nitrogen of the feces of his nitrogen-free diets as a part of the endogenous whole. If the previously discussed view (Sec. 93) that the metabolic nitrogen is a function of the digestive process primarily is accepted, the ratio may be more appropriately calculated on the basis of the urinary nitrogen only, considering that the latter is more truly representative of the same vital processes which are responsible for the energy catabolism. Using this basis, Smuts<sup>2</sup>

<sup>1</sup> TERROINE, E. F., and HÉLÈNE SORG-MATTER, Loi quantitative de la dépense azotée minima des homéothermes: validité intraspécifique, *Arch. intern. physiol.*, **29**, 121-132, 1927.

<sup>2</sup> SMUTS, D. B., The relation between the basal metabolism and the endogenous nitrogen metabolism, with particular reference to the estimation of the maintenance requirement of protein, *J. Nutrition*, **9**, 403-433, 1935.

has confirmed the work of Terroine as to the approximate constancy of the ratio among different species. Additional evidence is also furnished by the recent work of Brody and associates<sup>1</sup> who further confirmed the relationship by showing that endogenous nitrogen is proportional to practically the same power of live weight as is basal metabolism.

A study of these various experiments is convincing that a relationship between the energy and nitrogen catabolism of fasting exists, and the present evidence indicates a ratio of approximately 2 mg. of nitrogen per Calorie. The variability of the data reported, however, suggests that further studies are needed to determine the degree to which the ratio may be considered constant, particularly for all ages and species. The same difficulties previously mentioned for the determination of a significant value for the minimum endogenous nitrogen obviously apply to the determination of the ratio. Since the measurement of the energy catabolism is subject to fewer uncertainties than is the case for nitrogen, the establishment of a reliable and generally applicable figure for the ratio would be a distinct advantage in that it would permit the calculation of the value for endogenous nitrogen from the more easily determined energy value.

**227. Mineral Catabolism in Fasting.**—From the discussion in Chap. VII of the functions of mineral elements, it is evident that an active mineral metabolism continues during fasting. Differing from organic constituents of the body, however, catabolized minerals may be reutilized instead of being excreted. For example, although the red cells of the blood are constantly being destroyed, the catabolized iron is available for the resynthesis of hemoglobin.

There is, nevertheless, a constant excretion of mineral elements during fasting as is evident from the study reported by Benedict.<sup>2</sup> He measured the urinary excretion of certain minerals by a man during a fast of 31 days. The outputs gradually decreased during the early days and then reached values which tended to become constant. The figures obtained for the last day's output, expressed in grams were as follows: chlorine, 0.13; phosphorus, 0.58; sulfur, 0.49; calcium, 0.138; magnesium, 0.052; potas-

<sup>1</sup> BRODY, PROCTER, and ASHWORTH, *loc. cit.*

<sup>2</sup> BENEDICT, FRANCIS G., A study of prolonged fasting, *Carnegie Inst. Wash., Pub.* 203, 247-291, 1915.



sium, 0.606; sodium, 0.053. The relatively large excretions of phosphorus and especially of sulfur doubtless were due in part to the breakdown of protein containing these elements as reflected by the endogenous nitrogen catabolism. This fact suggests that the data cannot be considered to indicate the catabolism of mineral fasting alone. Had there been an adequate intake of energy and protein, certainly less sulfur- and phosphorus-containing protein would have been broken down. The large output of potassium in contrast to the low excretions of sodium, calcium, and chlorine is less readily explainable. These data from Benedict do not measure the total excretion of all the minerals considered since the feces are an important path of outgo for some of them.

#### MAINTENANCE REQUIREMENTS

The term requirement implies an exactness which it does not have, as frequently employed, and which it cannot have when used in feeding standards for practice. Strictly speaking, it is the minimum amount of a given nutrient needed to promote a given body function to the optimum in a ration optimum in all other respects; *i.e.*, a perfectly balanced ration. Such a minimum value will not be the same for any two individuals, and thus, for this reason alone, any determined individual value or any average of such values must be increased as a practical recommendation in order that the optimum performance of all may be assured. Further, perfectly balanced rations cannot be guaranteed in practice. For these and other reasons, the requirements specified in feeding standards must be higher than the minimum values experimentally determined under optimum conditions. Recognizing this fact Sherman has suggested the term, "allowance," in place of requirement in human dietary standards, but the term, requirement, appears to remain fixed in animal nutrition, though it has a varying significance.

Though not directly applicable to practice, the determination of the exact, minimum requirement is highly useful because it provides a baseline for studying the influence of factors which increase it in practice and thus for arriving at safe and yet economical recommendations as guides for feeding operations.

**228. The Maintenance Requirement for Total Nutritive Energy.**—The energy requirement for maintenance is the minimum amount needed to keep the animal in energy equilib-

rium; *i.e.*, to prevent any loss from its tissues. Thus an intake sufficient to offset the loss represented by the fasting catabolism would be the requirement under the conditions specified for measuring the latter. Expressed as net energy, it would be represented by the fasting catabolism itself, but, expressed as any other measure of total nutritive energy, it would obviously be larger, since no other measure represents energy which is completely utilizable for the prevention of tissue breakdown. For example, in translating the fasting catabolism into metabolizable energy, it would be necessary to choose a value for the latter which, minus its portion lost as heat, would equal the fasting value.

### 229. Basal Metabolism as a Measure of Energy Maintenance.

Under conditions of practice, however, an intake of total nutritive energy equivalent to the fasting catabolism is not an adequate maintenance value because the animal is never so restricted in its activity as represented by the standard, confined conditions which are specified for the determination of the basal value. The Report of the Conference on Energy Metabolism<sup>1</sup> defines the latter as a *physiologic* maintenance requirement which must be increased by an activity factor to provide the *economic* requirement for particular conditions of practice. At the present time there are no adequate experimental data for deciding what this activity factor should be. Clearly, it must vary for different animals and conditions and any value selected for general use needs to be high enough to cover the extreme cases in order to arrive at a maintenance figure which would be adequate under all conditions of practice. The same principle applies, however, to the formulation of any generalized feeding standard. Mitchell and coworkers<sup>2</sup> increased the basal metabolism values determined on chickens of different ages by 50 per cent to obtain their maintenance requirements in terms of net energy, on the basis that the added 50 per cent should cover the activity increment. Mitchell

<sup>1</sup> Report of the Conference on Energy Metabolism, held at State College, Pa., June 14-15, 1935, under the auspices of The Committee on Animal Nutrition of the National Research Council, Washington, D. C.

<sup>2</sup> MITCHELL, H. H., L. E. CARD, and T. S. HAMILTON, The minimum nutritive requirements of single comb white leghorn chickens, *Proc. 4th World's Poultry Congress, Sec. B, Nutrition and Rearing, Paper 49*, 301-306. 1930.

tentatively suggests the same percentage figure for arriving at the maintenance requirement of pigs, and 25 per cent as to the increment to be used for sheep and cattle.

Brody and coworkers<sup>1</sup> have published a set of maintenance values calculated from basal metabolism data and expressed in terms of total digestible nutrients. From a large number of data in the literature on the basal metabolism, they arrived at the following equation as applicable to all mammals:

$$\text{Basal metabolism (in Calories)} = 70.5M^{0.734}$$

$M$  is weight in kilograms. From a consideration of data of feeding trials, they decided that maintenance needs would be satisfied by providing an intake of total digestible nutrients equal to twice the basal metabolism. Hereby provision was made for both the activity factor and the losses undergone by digested nutrients in metabolism. Thus they multiplied the basal metabolism values for different weights by two to obtain the maintenance requirements as digestible energy. These values were translated into pounds of total digestible nutrients by dividing them by the figure 1812. This figure is based on the assumed relation: 1 g. of total digestible nutrients equals 4 Cal., whence 1 lb. equals 1812 Cal. In this way a table of maintenance values for various weights ranging from 0.1 to 1000 kg., and from 0.1 to 2000 lb. was constructed. The values take account of the fact that the principal energy expenditure of maintenance is proportional to a fractional power of weight rather than directly proportional to it. Herein lies an improvement over standards which provide for the calculation as a direct relation. On the latter basis calculated values for weights markedly above the base value are unnecessarily high, while those for weights markedly below are inadequate. It is not certain, however, that the activity factor increases with the same fractional power of weight as does basal metabolism.

While it may be considered that the question as to the lability of basal metabolism and the lack of data as to the activity factor militate against the reliability of any maintenance values at present obtainable from fasting catabolism data, it is clear that further studies of this method are worth while.

**230. Determination of Maintenance Requirement from Feeding Trials.**—The maintenance values of feeding standards now in

<sup>1</sup> BRODY, PROCTER, and ASHWORTH, *loc. cit.*

common use have been arrived at by feeding trials. In its simplest form this method involves the determination of the amount of food required to hold adult animals at constant weight. The inclusion of a digestion trial in the course of the experimental period allows the expression of the requirement in terms of total digestible nutrients, or the latter may be calculated from the average coefficients. It is also possible, of course, to calculate the results to metabolizable energy. In such an experiment, in which live weight is the sole criterion, the importance of accurate and representative data for this measure is clear. If the experiment is successful in maintaining the weight substantially constant over an extended period, a fairly accurate measure of the maintenance requirement is obtainable and a measure which is directly applicable to the conditions of practice. Allowances can be made for changes in live weight by estimating the food equivalent of the losses or gains and correcting the observed intakes accordingly. Such corrections can be only approximate at best because of a lack of knowledge of the kind of tissue gained or lost. As an extreme example, the change in weight might be due entirely to water, which of course would have no food equivalent at all. It is clear that the larger the corrections which have to be applied the less significant become the results.

The preceding considerations indicate the basis of a more general criticism of the live-weight method, *viz.*, that constancy of weight does not necessarily imply the maintenance of the integrity of the body tissues or a constancy of energy content. In the case of a young animal, for example, in spite of a constant weight, an increase in protein and minerals may take place, representing a growth the energy for which is furnished in part by a catabolism of body fat. This uncertain feature of the feeding-trial method can be eliminated by including a slaughter test, as is illustrated by the work of Mitchell and coworkers<sup>1</sup> with lambs. In this experiment a check group was slaughtered at the start, and the experimental group was slaughtered at the close of the period during which the food intake for maintenance was determined. A comparison of the slaughter data for the two lots showed that the experimental lot actually gained in energy and a correspond-

<sup>1</sup> MITCHELL, H. H., W. G. KAMMLADE, and T. S. HAMILTON, A technical study of the maintenance and fattening of lambs and their utilization of a ration of alfalfa hay and corn, *Ill. Agr. Expt. Sta. Bull.* 314, 1928.

ing deduction was accordingly made in the observed food intakes in arriving at the maintenance requirement. The experiment included a digestion and metabolism trial which enabled the expression of the results both as total digestible nutrients and as metabolizable energy. This method has also been used in studies with swine.

The inclusion of the slaughter procedure adds much to the accuracy of the results, but it also adds greatly to the cost of the determination and is not practicable for all classes of stock.

**231. The Determination of Energy Equilibrium.**—The use of a respiration apparatus or respiration calorimeter makes possible the measurement of the effectiveness of a given ration for the maintenance of tissue integrity without slaughter of the animals. This procedure was early used by Kellner, Armsby, and others as a basis for obtaining the maintenance requirement. It involves the determination of the energy balance with a ration which is just adequate to maintain weight. It cannot be expected that any such ration will result in exact energy equilibrium, but the procedures furnish specific data as to any tissue gains or losses, and the feed energy intake, whether expressed as digestible, metabolizable, or net, can be corrected accordingly to arrive at the exact maintenance requirement. Kriss<sup>1</sup> has cited findings of the Institute of Animal Nutrition that the average heat production of the seven cows in energy equilibrium on a normal mixed ration was 8.487 Therms of metabolizable energy per 1000 lb. live weight. This maintenance requirement was translated into digestible nutrients by dividing by the factor 1.616 (1 lb. total digestible nutrients is equivalent to 1.616 Therms metabolizable energy).

The energy-equilibrium method of determining the maintenance requirement is recognized in the Report of the Conference on Energy Metabolism<sup>2</sup> in the following words:

The standard *physiologic* maintenance requirement of an animal for net energy is that quantity necessary for the maintenance of energy equilibrium under ideal conditions, computed to a day of 12 hours

<sup>1</sup> KRISS, MAX, A comparison of feeding standards for dairy cows, with special reference to energy requirements, *J. Nutrition*, 4, 141-161, 1931.

<sup>2</sup> Report of the Conference on Energy Metabolism, held at State College, Pa., June 14-15, 1935, under the auspices of The Committee on Animal Nutrition of the National Research Council, Washington, D. C.

standing and 12 hours lying. The standard conditions of environment are as in the respiration calorimeter or chamber, with the air temperature neither below the critical nor above the point of hyperthermal rise. The *economic* maintenance requirement must include an additional quota of energy sufficient to cover the energy expenditure in muscular activity under the particular conditions prevailing.

Forbes and Kriss recognized the need for this additional quota in connection with their standard<sup>1</sup> for dairy cows when stating that their values determined in the respiration calorimeter would presumably need revision upward to be applicable in practice.

TABLE XXI.—SOME MAINTENANCE VALUES OBTAINED BY VARIOUS METHODS

Method	Species	Body weight, lb.	Total digestible nutrients, lb.	Metabolizable energy, Therms	Net energy, Therms
Fasting metabolism	All	1000	6.75 (B)		4.08 (ZH) 0.141 (MCH)
	Horse	1000			
	Pullet	4			
Energy balance	Dairy cow	1000	5.51 (FK)	8.487 (FK) 1.32 (A) 11.9-13.1 (ZH)	5.5 (C) 6.0 (A) 0.72 (A)
	Steer	1000			
	Sheep	100			
	Horse	1000			
Live weight	Dairy cow	1000	6.48 (H) 5.97 (FK) 7.925 <sup>1</sup> (Ha)	1.37 (A)	0.79 (A)
	Dairy cow	1000			
	Dairy cow	1000			
	Sheep	100			
Live weight and slaughter	Beef cow	1000		12.92 (TMH)	
	Sheep	100		1.64 (MKH)	
	Pig	100		1.86 (MH)	

The letters given in parentheses refer to the investigators as follows: A, Armsby; B, Brody; C, Cochrane, Fries, and Braman; FK, Forbes and Kriss; H, Hills; Ha, Haecker; MCH, Mitchell, Card, and Hamilton; MH, Mitchell and Hamilton; MKH, Mitchell, Kammlade, and Hamilton; TMH, Trowbridge, Moulton, and Haigh; ZH, Zuntz and Hagemann. Their publications have been previously cited or are to be found at the end of the chapter.

Some maintenance values obtained for different species by the various procedures previously discussed are given in Table. XXI. These data are presented to indicate the relative magnitude of the requirements as expressed in different ways and as arrived at by

<sup>1</sup> KRISS, *op. cit.*

different procedures. It is noted that the values are given in terms of specific body weights. The original publications list a series of corresponding values for various weights or indicate how they may be obtained. The earlier experiments dealing with the maintenance requirement are extensively reviewed by Armsby.<sup>1</sup> Further information is presented by Morrison<sup>2</sup> who gives a standard for dairy cows ranging in weight from 700 to 1800 lb., expressed in terms of both total digestible nutrients and net energy.

**232. The Protein Requirement for Maintenance.**—The discussion in Chap. VI has shown that the need of the body for nitrogenous food, which we commonly refer to as a protein requirement, is actually a need for the building stones of protein, *viz.*, the amino acids. It was also brought out in that chapter that the figures for the protein content of foods are conventional values, calculated from nitrogen content, and thus that they include both proteins and other nitrogenous compounds calculated to a protein basis. Despite these limitations, we express the nitrogen phase of nutrition on a protein basis because it is simpler to do so, and because our knowledge is insufficient for a more exact expression. No serious disadvantage is here involved provided the limitations are kept in mind.

**233. Biological Value and Protein Requirement.**—While the nitrogen requirement of the body for a given function may be absolutely fixed, the amount of digested food protein which will satisfy this requirement is variable according to its amino-acid make-up. No protein combination has a 100 per cent biological value, even though its amino-acid mixture exactly corresponds to what is needed, because some deaminization and oxidation always occur. This breakdown tends to increase with the level of intake. Thus there can be no fixed minimum value for protein requirement for any body function, except in terms of a specific protein or combination, and any requirement which is made the basis of a general feeding standard, applicable to any and all combinations of feeds, must be high enough to be adequate with the ration of poorest biological value. Therefore, though specific

<sup>1</sup> ARMSBY, HENRY PRENTISS, *The nutrition of farm animals*, The Macmillan Company, New York, 1917.

<sup>2</sup> MORRISON, F. B., *Feeds and feeding*, Morrison Publishing Company, Ithaca, N. Y.



minimum requirements are important experimentally, a protein allowance for general application must be a value which can be counted upon to be adequate without being unnecessarily high, rather than a minimum figure. Lower figures can be used for specific combinations which have been studied or for which some estimate of their biological value can be formed.

The constant loss of nitrogen which occurs on a nitrogen-free, energy-adequate diet bears witness to the body's need for nitrogenous food to maintain the integrity of its tissues. This endogenous nitrogen comes, at least in part, from simpler nitrogenous compounds than tissue proteins. Thus the requirements for maintenance are somewhat different from those of growth, and they may be of a simpler order. There is considerable evidence for this viewpoint, as is illustrated by the experiment of Osborne and Mendel<sup>1</sup> showing that for growth zein required the addition of both tryptophane and lysine, but that for maintenance tryptophane only was needed. McCollum and Steenbock<sup>2</sup> found that incomplete proteins were highly effective in partially replacing the nitrogen losses in pigs on a nitrogen-free diet. Other workers have reported that nitrogenous compounds simpler than amino acids, *viz.*, asparagine and other amides, have been found effective in decreasing the loss of endogenous nitrogen. These experimental observations must not be interpreted to mean that the nature of the dietary nitrogen is of no importance in maintenance. There is ample evidence that, for the attainment of complete nitrogen equilibrium, the quality of the protein intake has an influence on the amount required. It seems probable that a part of the maintenance requirement is for relatively simple body constituents which can be supplied by incomplete proteins and even by amides, but that complete maintenance involves also the synthesis of proteins needed for such purposes as the secretion of digestive juices and hormones, and that dietary protein containing all of the essential amino acids is here demanded. Thus the quality or biological value of the nitrogen intake is important in maintenance as well as for growth, though probably not to the same degree. A stated protein requirement for maintenance

<sup>1</sup> OSBORNE, THOMAS B., and LAFAYETTE B. MENDEL, Amino acids in nutrition and growth, *J. Biol. Chem.*, **17**, 325-349, 1914.

<sup>2</sup> MCCOLLUM, E. V., and H. STEENBOCK, Studies of the nutrition of the pig, *Wis. Agr. Expt. Sta. Research Bull.* 21, 1912.



assumes that the ration is adequate as regards energy so that protein can serve its specific purpose.

Morris and Wright<sup>1</sup> have studied the relation of protein quality to the maintenance requirement for cattle. They conclude that tryptophane, arginine, and histidine are always present in roughage in adequate amounts and that any requirement for cystine is very small, but that special attention must be given to lysine. They estimate the lysine requirement as 0.8 g. of lysine nitrogen per 1000 lb. live weight.

**234. Estimation of Protein Requirement from Endogenous Nitrogen.**—The endogenous nitrogen can be used as a measure for the body nitrogen which the food protein must spare in protein maintenance, provided certain assumptions are made as to the losses in metabolism. Various workers have proposed figures for maintenance requirement on this basis. Mitchell<sup>2</sup> came to the conclusion that an endogenous nitrogen value of 0.030 g. per kilogram body weight daily was a fair figure to apply to all species. Considering that commonly fed rations should have a biological value of at least 50 per cent in meeting this endogenous loss, he suggested a maintenance requirement of 0.06 g. of digestible nitrogen per kilogram, or approximately 0.4 lb. digestible protein per 1000 lb. live weight.

As a result of studies of the relation of endogenous nitrogen to basal metabolism Smuts<sup>3</sup> working in Mitchell's laboratory has suggested an estimation of the maintenance requirement on this basis. Using Brody's equation (Sec. 229) for the relation of basal metabolism to body weight and considering that 2 mg. of nitrogen or 12.5 mg. of protein is required per Calorie of basal heat, the following formula is proposed for the protein requirement:

$$P = 0.88M^{0.734}$$

where  $P$  is the day's protein requirement expressed in grams and  $M$  the body weight in kilos. Such a value obviously refers to protein actually utilized in the replacement of endogenous

<sup>1</sup> MORRIS, SAMUEL, and NORMAN C. WRIGHT, The nutritive value of proteins for maintenance, *J. Dairy Research*, 6, 289-302, 1935.

<sup>2</sup> MITCHELL, H. H., The minimum protein requirements of cattle, *Nat. Research Council Bull.* 67, 1929.

<sup>3</sup> SMUTS, *loc. cit.*

nitrogen and must, therefore, be increased to allow for wastage in metabolism. Considering that the protein of commonly fed rations should have a biological value of at least 50, the actual requirement was obtained by doubling the value calculated from the formula. This method takes account of the fact that the requirement is proportional to a power of weight. For a 1000-lb. animal, it figures out as 0.35 lb. of digestible protein.

While it is agreed that endogenous nitrogen provides the basic value for arriving at the maintenance requirement, the uncertainties as to the correctness of present values, *viz.*, as to whether they are actually minimum values and as to whether they represent the actual catabolism under maintenance conditions, suggest that any requirement so arrived at must be thoroughly tested in practice before it can be accepted. The same reservation applies to calculations based on the ratio of endogenous nitrogen to basal metabolism. Such a ratio can be only as reliable as the endogenous nitrogen value, and present data on the ratio are by no means uniform. A further reason for testing such calculated values in practice is that they are much lower than the values determined from feeding trials, as is discussed later.

The studies of endogenous nitrogen have taught us that all species should have the same maintenance requirement for protein, in terms of a specific ration, per unit of body size. They have also suggested that the unit should be surface, or the corresponding fractional power of body weight. This concept is now being adopted in feeding standards for animals.

**235. Nitrogen-balance Data as a Measure of Protein Maintenance.**—The minimum protein intake in a ration otherwise complete, which will keep an animal in nitrogen equilibrium, is an exact measure of the requirement for the protein mixture in question. The *minimum* intake essential for equilibrium must be determined because in the adult animal in good nutritive condition there is no marked storage (Sec. 95) and thus an unnecessarily high intake tends to result in equilibrium also. The value obtained in a nitrogen-balance study of a specific food or ration is not a safe general recommendation because of differences in protein quality, and also because of the uncertainty as to whether such a value obtained over the short experimental period commonly involved can be considered the optimum for continuous feeding. The nitrogen-balance method is illustrated by the study

of Sherman<sup>1</sup> in arriving at his recommended protein allowance for man. From a consideration of the data of 109 published experiments, he found the average intake required for nitrogen equilibrium to be 45 g. daily, when calculated to a body weight of 70 kg. Recognizing the desirability of increasing this figure in arriving at an allowance for practice, his standard for adult maintenance recommends an intake of 1 g. per day per kilogram of body weight, an increase of approximately 50 per cent over the average figure required for equilibrium. From nitrogen-balance studies with the horse, Nitsche<sup>2</sup> has reported a maintenance requirement of 42 g. digestible protein per 100 kg. live weight.

**236. Determination of Protein Maintenance from Feeding Trials.**—The protein requirements which are used in most of the feeding standards at the present time are based upon the results of feeding trials, representing intakes, in rations considered otherwise satisfactory, which were found adequate for keeping the animals in good condition without loss of weight. Scientifically, the maintenance of weight and condition is no certain measure of the integrity of the nitrogenous tissue, or of the minimum requirement for this purpose, but the rations which prove satisfactory for such maintenance over extended periods are considered to supply an amount of protein which is at least adequate. It is evident that the inclusion of slaughter data in feeding trials provides for a more accurate measure of protein requirement than do observations on weight and condition alone.

Protein maintenance requirements for farm animals find their principal use in practice for dairy cows, and thus most of the feeding trials designed to determine them have been carried out with this species. From an extensive study of the data from these trials carried out prior to 1917, Armsby<sup>3</sup> came to the conclusion that an intake of 0.6 lb. of digestible protein per 1000 lb. live weight was an adequate allowance. While some of the later trials by other investigators have resulted in somewhat higher values, the most extensive study of all, by Hills,<sup>4</sup> resulted in the conclusion that the Armsby figure provides a sufficient intake. Morri-

<sup>1</sup> SHERMAN, H. C., Protein requirements of maintenance in man and the nutritive efficiency of bread protein, *J. Biol. Chem.*, **41**, 97-109, 1920.

<sup>2</sup> NITSCHKE, HERBERT, Eiweiss-minimum-bestimmung bei Arbeitspferden in Ruhe, *Landw. Jahrb.*, **82**, 429-438, 1936.

<sup>3</sup> ARMSBY, *loc. cit.*

<sup>4</sup> HILLS, J. L., C. L. BEACH, A. A. BORLAND, R. M. WASHBURN, G. F. E.

son has adopted this figure as the minimum allowance in his standard.

This value of 0.6 lb. per 1000 lb. is one-third or more higher than the previously mentioned figures suggested by data obtained from measurements of the endogenous nitrogen and basal metabolism. Without doubt it is unnecessarily high, at least with certain rations, but a sound recommendation for general practice must allow a sufficient margin of safety to cover rations of low biological value and to meet the needs of all individuals. Further, the minimum intake which is adequate in terms of the specific functions of protein may not be the optimum from the standpoint of the most efficient utilization of the ration as a whole (Sec. 218). For these reasons the allowance of 0.6 lb. per 1000 lb. body weight is considered a safer guide for practice at present than the lower values which in certain respects may have a more exact basis.

**237. Does the Protein Requirement for Maintenance Remain Constant during Production?**—The Folin theory (Sec. 97) postulates a constant endogenous catabolism of nitrogen independent of the total protein catabolism, and the theory implies that there is a constant requirement to meet this loss, irrespective of the protein metabolism which may be taking place for the support of other body functions. For example, the feeding of protein to meet the needs of such a function as growth does not alter the amount required for maintenance where the latter alone is involved. Many do not agree with this concept, basing their objection on the view that the maintenance requirement is for certain amino acids only and that on a nitrogen-free diet the catabolism of body nitrogen compounds to furnish the acids needed results in "leftovers" which are wasted. It is argued that during protein ingestion, on the other hand, this wastage is decreased. Arguments for this viewpoint have been set forth by Osborne and Mendel<sup>1</sup> and by Sherman<sup>2</sup> and have been critically reviewed by Mitchell and Hamilton.<sup>3</sup> Further evidence is discussed later (Sec. 321).

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STORY, and C. H. JONES, The protein requirements of dairy cows, *Vt. Agr. Expt. Sta. Bull.* 225, 1922.

<sup>1</sup> OSBORNE, and MENDEL, *loc. cit.*

<sup>2</sup> SHERMAN, *loc. cit.*

<sup>3</sup> MITCHELL, H. H., and T. S. HAMILTON, The biochemistry of the amino acids, Chemical Catalog Company, Inc., New York, 1929. Chap. IX.

The Folin theory is difficult to prove or disprove experimentally. Objections to its applicability in connection with the determination of the protein maintenance requirement relate primarily to the estimation of this requirement from endogenous nitrogen. The questions involved are of minor importance from the standpoint of feeding practice. It may be agreed that amino acids which are unsuitable or unneeded for a given productive function may serve in maintenance and thus lessen the specific intake for this purpose, but whether the gain here should be subtracted from the maintenance requirement or from the production requirement is a matter of bookkeeping.

**238. Mineral Requirements for Maintenance.**—The discussion in Chap. VII has shown that many of the mineral elements undergo a very active metabolism in connection with various processes which are essential for the normal functioning of the body in maintenance. Differing from the energy and protein metabolism, however, they are not necessarily used up and excreted in the process. Chlorine which is secreted in the gastric juice to provide for the digestion which is essential to life can be reabsorbed from the digestive tract and reutilized. The iron-containing red cells are being constantly destroyed but the iron so catabolized can be used again for hemoglobin synthesis. While both sodium and chlorine perform many functions in maintenance, experiments have repeatedly shown that on a salt-free diet the excretion of these elements nearly ceases. That there always is some loss of mineral elements during maintenance is evident from the data given for the fasting catabolism (Sec. 227). The extent to which a given mineral is excreted and to which, therefore, it is required in the maintenance ration is doubtless governed by variable factors such as body activity, nature of the mineral relations in the diet, and others. These variables suggest that there can be no fixed minimum requirement.

Of the dozen mineral elements which are essential for normal body function, it is recognized that only a few are ever sufficiently likely to be lacking in the commonly fed rations to merit attention as regards their requirements. Little study of the maintenance requirements of farm animals for even these few has been made, because these animals are fed primarily for productive purposes and there is little or no advantage in attempting to distinguish the relatively small maintenance requirements from

the total amounts needed. Much more attention has been given to the requirements in man.

Sherman<sup>1</sup> has formulated standards for the calcium and phosphorus requirements of man based upon a review of balance experiments. A study of 97 experiments showed calcium outputs ranging from 0.27 to 0.82 g. per man per day, with an average of 0.45 g. A study of 95 phosphorus balances showed outputs ranging from 0.52 to 1.20 g., with an average of 0.88 g. Sherman increased these average values by 50 per cent as a factor of safety in arriving at his suggested allowances of approximately 0.7 g. of calcium and 1.3 g. of phosphorus for the maintenance of the adult man (70 kg.). While the body contains more calcium than phosphorus, the larger percentage of the latter which is in the fluids and active tissues explains its larger requirement for maintenance.

Huffman and associates<sup>2</sup> have suggested a figure of 10 g. of phosphorus per 1000 lb. of live weight as an adequate maintenance intake for dairy cattle. Probably 8 g. of calcium should suffice for the same weight.

Iron is never lacking in the maintenance rations of farm animals except in very unusual situations (Sec. 122). No quantitative statements of its needs are available. In man the iron requirement has been variously estimated at from 0.07 to 0.13 mg. per kilogram. From the most recent work it appears that the lower figure represents a sufficient intake from a well-utilized source.

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<sup>1</sup> SHERMAN, H. C., Calcium requirement of maintenance in man, *J. Biol. Chem.*, **44**, 21-27, 1920; Phosphorus requirement of maintenance in man, *ibid.*, **41**, 173-179, 1920.

<sup>2</sup> HUFFMAN, C. F., C. W. DUNCAN, C. S. ROBINSON, and L. W. LAMB, Phosphorus requirement of dairy cattle when alfalfa furnishes the principal source of protein, *Mich. Agr. Expt. Sta. Tech. Bull.* 134, 1933.

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## CHAPTER XIII

### GROWTH

Growth is such a universal phenomenon that it commonly incites little curiosity in the layman; but when the physiologist faces the question, "what is growth?," he is overwhelmed by its complexities. The fertilization of a single cell starts a multiplication and a differentiation which become highly varied in kind and rate in the differentiated cells, yet remain coordinated and culminate in the adult. There is no complete explanation as to why the process starts, or how it is coordinated during its course, or why it stops at the definite point which characterizes adult development. As expressed by Rubner:<sup>1</sup>

Throughout the animate kingdom, from the simplest microorganisms to the most complexly organized beings, that inexhaustible power of growth which ever since the genesis of the first protoplasm in the infinite past has created the structure of the fossil remains of former ages as well as our own existence—this capacity to grow, has remained as the most remarkable phenomenon of nature, the supreme riddle of life.

Despite the complexities involved, physiological studies have produced a large body of information regarding the major processes of growth and some knowledge of these facts is obviously essential for an understanding of the nutritive requirements involved and as to how they can be met.

#### THE PHYSIOLOGY OF GROWTH

**239. The Nature of Growth.**—Clearly a process as complex as growth cannot be simply defined. It is much more than an increase in size. Schloss<sup>2</sup> defines growth as a "correlated increase

<sup>1</sup> RUBNER, MAX, *Das Problem der Lebensdauer und seine Beziehung zum Wachstum*, p. 81, R. Oldenbourg, Munich, 1908. Translation given in: MENDEL, LAFAYETTE, B., *Abnormalities of growth*, *Am. J. Med. Sci.*, 153, 1-20, 1917.

<sup>2</sup> SCHLOSS, ERNST, *Pathologie des Wachstums*, p. 4, S. Karger, Berlin, 1911.



in mass of the body in definite intervals of time, in a way characteristic of the species." This brief statement is excellent because it has very broad implications. It implies that, subject to individual variability, there is a characteristic rate of growth for each species and a characteristic adult size and development. It is considered that the maximum size and development are fixed by heredity. Nutrition is an essential factor determining whether this maximum will be reached, and an optimum nutritional regime is one which enables the organism to take full advantage of its heredity. According to the basic concept, however, the maximum development fixed by heredity cannot be exceeded through nutrition or by any other means, in the normal organism. The definition by Schloss also implies that in the growth of the organism as a whole there must be a complete and coordinated growth of all its parts. This simply stated characteristic involves a multitude of interrelated processes which are very imperfectly understood at the present time.

Optimum growth should result in an adult organism capable of optimum performance throughout its normal life. This is an extension to the life span as a whole of the previous statement that an optimum development is one which enables the organism to take full advantage of its heredity. Here again we are ignorant of many of the factors concerned, but it is evident that optimum growth in this sense includes much more than the rate of increase of weight and size.

True growth involves an increase in the structural tissues such as muscle and bone, and also in the organs. It should be distinguished from the increase that results from fat deposition in the reserve tissues. Thus growth is characterized primarily by an increase in protein, mineral matter, and water. From the nutritional standpoint, it involves in addition a large intake of energy-producing nutrients to support the growth processes, and an adequate supply of the various vitamins concerned is also required. A minute amount of lipid material goes into the structure of each cell, but this does not represent a specific dietary requirement with the possible exception of one or two fatty acids (Sec. 73), in view of the synthesis of lipid from carbohydrate.

**240. The Cell, the Unit of Growth.**—Growth takes place both by means of an increase in the number of cells, *hyperplasia*, and also through an increase in their size, *hypertrophy*. In early

embryonic life both processes occur in the case of all cells. In the adult three types of cells are differentiated: the *permanent* cells, such as those in the nerves, which ceased to divide early in prenatal life and whose number has remained fixed thereafter; the *stable* cells, including those of most organs, which continued to divide for a variable but major part of the growth period but which have become fixed in the adult; the *labile* cells, composing the epithelial and epidermal tissues, which continue to divide throughout life, the process in the adult being limited to the replacement of cells worn out. All of these three types of cells undergo hypertrophy during growth and some of them may increase in size thereafter in accordance with special physiological demands. For example, the increased muscular development which can be brought about through exercise involves a hypertrophy. The cells of the adult kidney can undergo enlargement if an increased burden is placed on this organ. It seems probable that the ability of the cells of the adult organism to hypertrophy becomes less with age.

**241. The Course of Growth of the Body as a Whole.**—Conception is the starting point of growth. The discussion in the present chapter deals with postnatal growth since it is more convenient to discuss intra-uterine growth as reproduction (Chap. XIV), but it should be remembered that the character of the latter has important bearings on the course of growth after birth. The evident vigor of the newborn, their content of certain nutrient reserves, and other qualities are influenced by the intra-uterine nutrition. This fact is reflected in the recognized desirability of considering the diet of the mother, and other factors affecting intra-uterine development, when selecting animals for many types of growth experiments. The percentage of the total growth period which is spent *in utero* differs in different species and this also has a bearing on the nutritional and other factors concerned in postnatal development. The longer the portion of the total period spent *in utero* the more advanced are the young at birth. The rat is born with its eyes closed, has no hair, does not gain the use of its legs for a considerable period, and must be nourished for a relatively long period solely by its mother's milk. In contrast, the guinea pig has a full coat of hair, its eyes are open at birth, and, within a few hours, it is running around nibbling leafy material. The calf, lamb, and foal resemble the guinea pig as

regards their stage of development at birth, while the pig and the human baby are more like the rat.

In the various species the time that is normally spent in growth bears a rather definite relation to the length of life. The data in Table XXII, calculated by Brody and associates,<sup>1</sup> give the age, in months from conception, at which definite percentages of the mature weight are achieved in the different species. While these figures would doubtless be modified if calculated from the more recent data on growth rates, they present a useful picture for comparative purposes.

TABLE XXII.—EQUIVALENCE OF GROWTH AGE

Species	Percentage of mature weight				
	10%	30%	50%	80%	98%
	Months	Months	Months	Months	Months
Holstein cow.....	10.6	16.0	23.4	43.3	93.2
Duroc-Jersey sow.....	5.9	10.1	15.7	31.0	67.3
Suffolk ewe.....	5.3	6.7	8.6	13.7	26.5
Guinea pig, male.....	2.8	3.9	5.3	9.3	18.9
White rat, male.....	2.1	2.8	3.6	5.9	11.7
White mouse, male.....	1.1	1.3	1.8	2.8	5.6

The rate of growth is not constant nor does its entire course follow any simple mathematical expression. There are periods of acceleration and of retardation. The observation of these periodic changes in rate of growth early led to the view that growth takes place in cycles. Robertson,<sup>2</sup> from a mathematical analysis of growth curves, came to the conclusion that in higher animals and man there are at least three definite growth cycles, of which the first is passed partially or wholly *in utero*. Robertson's views have been repeatedly challenged, but it is agreed that acceleration and retardation occur. In the human, for example, the curve of growth is characterized by a decreasing rate during

<sup>1</sup> BRODY, SAMUEL, CHESTER D. SPARROW, and HUDSON H. KIBLER, Time relations of growth. II. The equivalence of age in mammals estimated on the basis of their growth constants, *J. Gen. Physiol.*, 9, 285-308, 1925.

<sup>2</sup> ROBERTSON, T. BRAILSFORD, The chemical basis of growth and senescence, J. B. Lippincott Company, Philadelphia, 1923.

childhood, an acceleration during adolescence, and a decreasing rate thereafter.

**242. The Growth of Parts.**—The growth of the body as a whole is a resultant of the simultaneous growth of its parts for which the individual rates are widely variable. The skeleton increases as a percentage of the total body weight for a short period after birth and then decreases on this relative basis. This means that skeletal development tends to precede muscle growth. The

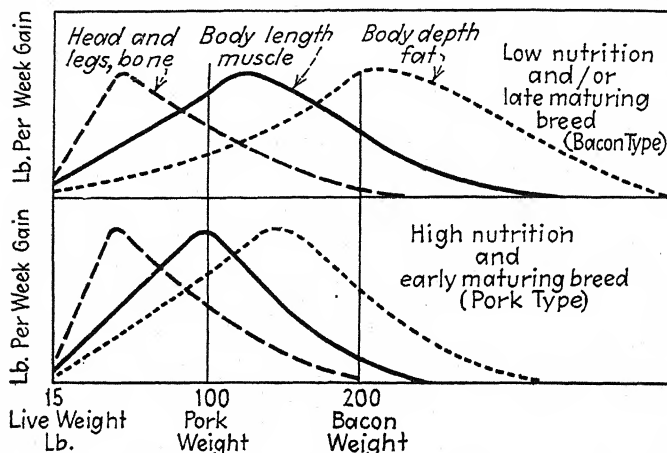


FIG. 26.—Rates of growth of parts. The curves portray the rates of growth for the parts considered. It is noted in both charts that the rate of growth for the head and legs reaches its maximum earlier than the rate for body length, and that the maximum for the latter is followed in turn by that for body depth. These three curves are considered to represent respectively: bone growth, muscle growth, and fat deposition. All of these processes occur earlier where the plane of nutrition is higher and earlier in the lard type of hog than the bacon type. (Courtesy of John Hammond, University of Cambridge.)

musculature increases as a percentage of body weight during growth, provided there is no marked fat deposition. There is always some deposition of fat during growth which becomes greater as maturity approaches. The head of the human baby is 25 per cent of its body size at birth, but only 7 to 8 per cent at maturity. These different growth rates for the various parts of the body explain the changes in conformation which take place as growth proceeds. Based upon his extensive studies, Hammond<sup>1</sup> has prepared the diagram shown in Fig. 26, illustrating

<sup>1</sup> HAMMOND, JOHN, Pigs for pork and pigs for bacon, *J. Roy. Agr. Soc.*, 93, 1-15, 1932.

how changes in body form and composition are brought about by differences in the time and rate of growth of different parts and tissues. Markedly different rates are exhibited by certain organs. The brain reaches adult size early in the growth period. The thymus increases to puberty and then decreases. The suprarenals actually lose weight for a time after birth, but this loss is balanced by an accelerated development toward the end of the growth period.

**243. Measures of Growth.**—The growth of the body as a whole is most commonly measured as an increase in weight. Size measures, such as height, and various other body dimensions are also frequently employed. A combination of both weight and size measures is much more useful than either alone. An animal may increase in weight through the deposition of fat without any increase in the structural tissues and organs which characterize growth. An animal which is receiving insufficient protein and energy, to permit of the growth of its muscles and organs, may still show an increase in size due to skeletal growth.

The increase in mass of the body as a whole may be expressed absolutely, as in grams per day, or it may be expressed as a percentage of the mass at the start. The absolute measure is the one most commonly employed in growth experiments, but the relative measure, which records the percentage increase, gives a more useful picture in many instances. The latter measure is plotted on semilogarithmic coordinate paper as is shown in Fig. 27. These curves illustrate the usefulness of the measure in comparing the growth rates of species of widely different size. Clearly the absolute increases in weight would be useless for such comparison.

Increase in weight and size are highly useful measures of growth but they are obviously incomplete. They do not show the nature of the tissue formed, nor are they suitable measures of coordinated development. The amounts of the true growth tissue, *viz.*, protein and the skeletal minerals can be obtained by a balance experiment (Sec. 194) or by the slaughter procedure (Sec. 183). The latter must be employed to record the growth of organs and other parts which provide measures of coordinated growth.

**244. "Normal Growth."**—In nutrition studies much use is made of "normal" growth data as illustrated by height-weight tables for children, and by curves for increase in weight and size

for farm animals such as those presented by Moulton and associates.<sup>1</sup> These data are useful for comparative purposes, but their limitations must be realized. They are averages of the increases found for a group of individuals for which the nutritive and other factors were considered to be adequate to produce a satisfactory adult. Since only the growth of the whole is measured, it cannot be said that the growth is coordinated or complete in all its aspects. It is unsafe to assume that this use of the term normal implies an optimum development.

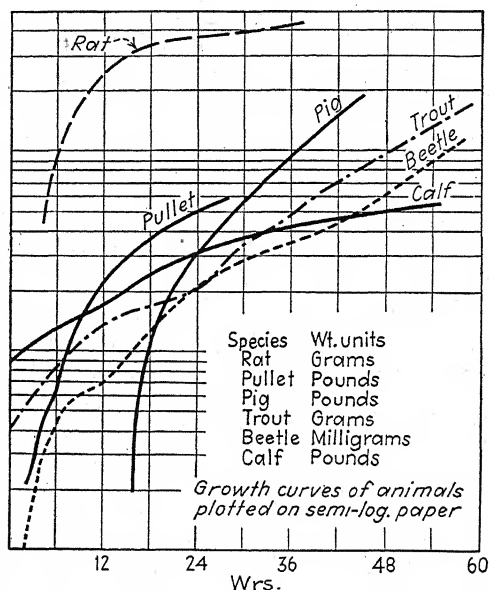


FIG. 27.—Growth of various species plotted on semilogarithmic coordinates. (Courtesy of C. M. McCay, Cornell University.)

**245. Internal Secretions and Growth.**—The striking abnormalities of growth which result when certain organs of internal secretion fail to function normally bear witness to the vital role of these secretions in controlling and coordinating the growth processes and furnish a further illustration of their complexity. Underdevelopment or hypoactivity of the thyroid stunts growth in size

<sup>1</sup> MOULTON, C. R., H. L. KEMPSTER, A. G. HOGAN, and SAMUEL BRODY, Normal growth of domestic animals, *Mo. Agr. Expt. Sta. Research Bull.* 62. 1923.

of the body as a whole with a relative overgrowth of certain parts, and mental development is arrested also, producing a condition which is called *cretinism*. In contrast, the overproduction of a secretion from the anterior lobe of the pituitary results in *gigantism*. There is an excessive skeletal growth which produces a giant in stature, but a weakling physically because other growth processes do not keep pace. When the pituitary gland is underactive, the individual is small and infantile in appearance. Other internal secretions also play roles in growth, and there are definite interrelations in the functions of certain of the endocrine glands in the coordinating of the processes which combine to produce normal development, physical, and mental.

**246. Retardation of Growth.**—The severe retardation or arrest of growth which results from a failure of glandular secretions is fortunately comparatively rare. Much more common are the lesser retardations which are caused by undernutrition, either in calories or in some specific essential nutrient. The nature and extent of the effect on growth are dependent upon the character and severity of the deficiency and upon the period involved. A deficiency of energy, for example, will immediately check growth in mass, while a lack of calcium may not, since its primary effect is upon bone structure rather than its size. A deficiency of certain other nutrients, such as phosphorus or vitamin B, exerts an indirect influence on increase in size by decreasing appetite, as well as causing direct physiological effects.

The influence of varying degrees of undernutrition on the growth process has been the object of much study. One of the pioneer studies was that of Waters<sup>1</sup> in which steers were placed on a ration which permitted no gain in weight, and the resulting changes in conformation were observed. Though the weight remained stationary, growth was not entirely inhibited, for the steers increased in length and height; but they became exceedingly thin because of a depletion of their fat reserves. Many experiments with laboratory animals, notably those by Osborne and Mendel<sup>2</sup> have shown that, following a period of retardation,

<sup>1</sup> WATERS, H. J., The capacity of animals to grow under adverse conditions, *Proc. Soc. Promotion Agr. Sci.* (29th ann. meeting), 1908, pp. 71-96; How an animal grows. The influence of nutrition on the size and form of animals, *Kan. State Board Agr. Rept.* 113, 59-85, 1910.

<sup>2</sup> OSBORNE, THOMAS B., and LAFAYETTE B. MENDEL, The resumption of

growth in weight can be resumed at a more rapid rate than normally exhibited at any time during life. There is no adequate explanation of this. In stunting, cells may be depleted yet remain in outline, capable of being filled in later without complete rebuilding. The rapid increase in weight which follows retardation may be to a considerable extent a replacement of lost fat and this process may take place more rapidly than true growth. The actual suppression of growth may be less than the weight measures indicate. Cellular development may proceed in important ways and yet not be reflected in any increase in weight.

The major interest in the effects of growth retardation lies in the question of its influence on ultimate body size and development. Does any prolonged or severe retardation permanently harm any tissues or functions? The view is commonly expressed that any marked retardation is definitely undesirable. This may be true, but experiments have shown that underfeeding may prolong the growth period without affecting the ultimate size. The previously mentioned, severe treatment to which steers were subjected by Waters did not result in any permanent stunting, according to later reports from the Missouri Experiment Station where the study was carried out. Of greater interest is the established fact that the growth impulse may be exercised in an important way long after the time of life at which growth is normally completed. Osborne and Mendel<sup>1</sup> found that the rat could resume growth following retardation, even though it had completed approximately three-quarters of his life span, and they considered that "full size" was thus attained. Later, in a more comprehensive experiment, McCay and associates<sup>2</sup> obtained a resumption of growth at much later ages than those tested by Osborne and Mendel, and this growth was reflected in organ development as well as in weight, but these retarded animals

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growth after long-continued failure to grow, *J. Biol. Chem.*, **23**, 439-454, 1915.

<sup>1</sup> OSBORNE, THOMAS B., and LAFAYETTE B. MENDEL, Acceleration of growth after retardation, *Am. J. Physiol.*, **40**, 16-20, 1916.

<sup>2</sup> McCAY, C. M., MARY F. CROWELL, and L. A. MAYNARD, The effect of retarded growth upon the length of life span and upon the ultimate body size, *J. Nutrition*, **10**, 63-79, 1935.



never reached a body size equal to that of animals allowed to grow without retardation.

**247. Accelerated Growth Performance.**—Mendel and Hubbell<sup>1</sup> have reported a very interesting study of the growth of the rat during three periods in the history of the inbred colony of the Connecticut Agricultural Experiment Station. A gradual increase in growth rate has occurred during the 25 years, and the animals of today are markedly larger as adults than those of 1912. Improvements in the nutritional regime are considered to be largely responsible for these developments. Similar data have been reported for man. There are several studies, such as those published by Appleton,<sup>2</sup> showing that Orientals grow more rapidly and to a larger size in a Western environment. Various factors, such as sanitary conditions and climate as well as nutrition, are doubtless concerned.

As summarized in a recent review,<sup>3</sup> there are convincing data from selected groups in this country and elsewhere that a physical evolution is occurring whereby children are growing to be taller and heavier than their parents. Better and more abundant food is listed as one of the causes.

These various results do not necessarily mean that the development fixed by heredity is actually being exceeded. Rather, they may mean that we are merely learning how to take fuller advantages of the hereditary capacity to grow and that the attainment of the maximum possible development lies in the future.

**248. Rate of Growth and Productive Life.**—Clearly the recent studies in nutrition have resulted in practices that have markedly increased the growth rate as measured by weight and size. It is the general belief that this is a desirable development in terms of the life span as a whole. Evidence for this viewpoint has been produced in extensive experiments with rats by Sherman and

<sup>1</sup> MENDEL, LAFAYETTE B., and REBECCA B. HUBBELL, The relation of the rate of growth to diet. III. A comparison of stock rations used in the breeding colony at the Connecticut Agricultural Experiment Station, *J. Nutrition*, **10**, 557-563, 1935.

<sup>2</sup> APPLETON, VIVIA B., Growth of Chinese children in Hawaii and in China, *Am. J. Phys. Anthropol.*, **10**, 237-252, 1927; Growth of Kwangtung Chinese in Hawaii, *ibid.*, **11**, 473-500, 1928.

<sup>3</sup> Editorial: Are we growing bigger? *J. Am. Med. Assoc.*, **107**, 1054-1055, 1936.

associates.<sup>1</sup> McCay and coworkers,<sup>2</sup> on the other hand, have challenged this dominant viewpoint in so far as length of life is concerned and have produced important evidence that animals made to grow slowly by calorie restriction have a longer life than those that grow very rapidly under ad libitum feeding.

These experiments serve to emphasize the fact that growth should be looked at primarily as a preparation for life. In man, we are interested in a healthy, productive life in which the infirmities of old age are postponed as long as possible. In breeding stock and in animals kept for milk and egg production lifetime performance is the final measure of the success achieved in rearing these animals. The possibility that, from the standpoint of productive life, there are limitations in a system of rearing which relies upon a rapid increase in weight and size as the primary measure of success has been discussed in Chap. I. However much one may doubt this possibility, it cannot be said with certainty that all the factors for growth are optimum until their influence on lifetime performance has been studied as thoroughly as their effects during the growth period itself.

#### TOTAL-NUTRITIVE-ENERGY REQUIREMENTS FOR GROWTH

Previous discussions have shown that the rate and character of the body increase vary with age, as well as with species. It is evident, therefore, that a feeding standard for growth must be different for each species and must consist of a series of values corresponding to the different ages and body weights representing the growth period. Such a detailed presentation for all species is beyond the scope of a text dealing with the principles of nutrition. Rather, the physiological bases of the requirements will be considered, typical procedures for arriving at specific values will be outlined, and reference will be made to sources of detailed information for the different species which have been studied.

<sup>1</sup> SHERMAN, H. C., Nutritional improvement in health and longevity, *Sci. Monthly*, **43**, 97-107, 1936. SHERMAN, H. C., and H. L. CAMPBELL, Rate of growth and length of life, *Proc. Nat. Acad. Sci.*, **20**, 413-416, 1934; Effect of increasing the calcium content of a diet in which calcium is one of the limiting factors, *J. Nutrition*, **10**, 363-371, 1935.

<sup>2</sup> McCAY, C. M., MARY F. CROWELL, and L. A. MAYNARD, *loc. cit.* McCAY, C. M., and MARY F. CROWELL, Prolonging the life span, *Sci. Monthly*, **39**, 405-414, 1934.

The total requirement for a given nutrient during growth must include the amount needed for maintenance as well as the amount required for the new tissue formed. The values given in feeding standards represent these combined requirements. Of the various nutrient needs for growth, the requirement for total nutritive energy is by far the largest and primarily governs the total food allowance. It is, therefore, advantageous to discuss this requirement first.

#### 249. Factors Governing the Energy Requirement for Growth.

The maintenance component of the total energy requirement during growth increases regularly with body size, but the additional demand for the growth itself varies with the rate and with the composition of the tissue formed. Per unit of body weight, the amount of energy represented by the growth tissue formed decreases with age, reflecting the declining rate of body increase measured on a percentage basis (Sec. 243). But the amount of energy stored per unit of body increase becomes larger with age because of its lower water and higher fat content. While the true growth tissue contains only a trace of fat, a certain amount of fat deposition is an inevitable accompaniment of growth, and in practice a considerable amount of fattening is an integral part of growing animals for meat. Since fat contains much more energy than does protein, it is evident that the energy requirement per unit of body gain increases in accordance with its fat content. In fact, if the fattening is very rapid, the normal trend of decrease in energy stored per unit of body weight may not occur. In feeding standards for meat animals, no separate statement is made of the requirements for growth proper and for the fattening which concurrently takes place, but a distinction may be made according to the amount of fattening desired.

Except under conditions where very rapid fattening is sought, the maintenance component of the total growth requirement always markedly exceeds the portion required for the formation of new tissue. Thus the faster the growth rate the lower the total requirement per amount of gain tends to be, but this tendency may be counterbalanced by the decreasing efficiency of food utilization as the intake is increased. In fact, the most rapid gains may be definitely uneconomical in terms of the total feed required. Ellis and Zeller<sup>1</sup> reported that restricting the

<sup>1</sup> ELLIS, N. R., and J. H. ZELLER, Effect of quantity and kinds of feed on

allowance of growing pigs to approximately three-quarters of "full feed" generally decreased the feed required per unit of gain, although the rate of gain was markedly lowered. In terms of energy stored, however, the savings made were subject to discount because the restricted levels tended to produce gains containing relatively less fat. From the standpoint of practice, the increased labor and other charges involved in the longer time required to bring the animals to market weight may offset the savings in feed costs. Rapid growth will get the animals to market earlier when prices may be better, but it will produce a carcass which may be discounted where a high proportion of lean is desired.

Since balanced rations (Sec. 218) involve less wastage as heat production, they have the practical effect of decreasing the total feed required per unit of gain. The economy of balancing the hog ration with respect to protein has been shown by Ellis and Hankins<sup>1</sup> in studies involving three levels of protein intake. The higher the level, the more economical were the gains in terms of total feed required. The differences were most marked during the early part of the growth period. Similar data have been obtained with chickens.

**250. Energy Requirements from Metabolism and Slaughter Data.**—The net-energy requirement for growth may be considered to be the sum of the energy of the tissue formed, plus the basal metabolism increased by an activity factor (Sec. 229). Mitchell<sup>2</sup> has presented net-energy requirements for the growth of steers, pigs, ewes, chickens, and rats based upon a calculation using data obtained in his own laboratory and elsewhere. Data from the slaughter and analysis of animals at different stages of growth provided the basis for calculating the energy content of tissue formed, and the basal metabolism figures were obtained from various experiments. Mitchell recognized that there was no accurate basis for arriving at the activity factor, but from avail-

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economy of gains and body composition of hogs, *U. S. Dept. Agr. Tech. Bull.* 413, 1934.

<sup>1</sup> ELLIS, N. R., and O. G. HANKINS, The influence of the protein content of the ration on the growth and fattening of hogs fed at a moderately restricted level, *Proc. Am. Soc. Animal Production*, 1935, pp. 107-111.

<sup>2</sup> MITCHELL, H. H., Relations between the metabolism of protein and of energy, *Proc. Am. Soc. Animal Production*, 1933, pp. 229-232.

able information he concluded that the average daily expenditure in muscular activity represents 25 per cent of the basal heat for cattle and sheep, 50 per cent for swine and chickens, and 5 to 10 per cent for rats.

The basic data and calculations for arriving at the net-energy requirement for growth by the above procedure are given in much more detail for certain species in earlier publications by Mitchell and his associates. As an example, the presentation for chickens by Mitchell, Card, and Hamilton<sup>1</sup> may be cited. The data in Table XXIII, taken from this bulletin, give the estimated daily requirements of White Leghorn cockerels, representing a rapidly growing species. The heading, "maintenance," denotes the basal metabolism. The data in the last column translate the total requirements into grams of corn assuming a net-energy value of 280 Cal. per 100 g. It is noted that the requirement for the tissue formed is much lower at the higher weights, reflecting the declining rate of increase, and that at its maximum it is less than half the basal metabolism and is exceeded by the activity factor.

TABLE XXIII.—NET-ENERGY REQUIREMENTS OF GROWING COCKERELS

Body weight, lb.	Net energy, Cal.				Equivalent weight of corn, g.
	Maintenance	Activity	Growth	Total	
0.5	37	18	15	70	25
1.0	55	27	19	101	36
1.5	59	29	21	109	39
2.0	72	36	21	129	46
3.0	94	47	19	160	57
4.0	114	57	14	185	66
5.0	133	66	10	210	75

Mitchell considers the data, such as those represented by the preceding table, to provide an illustration of a method of computing requirements rather than as a finished statement of them. He recognizes that they should not be adopted for practice until tested by appropriate feeding trials and modified accordingly. Their utilization in practice is subject to the difficulties and

<sup>1</sup> MITCHELL, H. H., L. E. CARD, and T. S. HAMILTON, A technical study of the growth of White Leghorn chickens, *Ill. Agr. Expt. Sta. Bull.* 367, 1931.

uncertainties involved in the use of the net-energy measure in evaluating feeds and in computing rations (Sec. 219).

**251. Energy Requirements Obtained from Feeding Trials.—**

The data contained in the currently used feeding standards for farm animals are based primarily on the results of feeding trials. In the more critically conducted trials different groups of animals have been fed throughout the growth period at different levels of energy intake to ascertain the level that would produce normal growth and development without being unnecessarily high. A feeding trial enables the statement of the requirement in terms of specific feeds or in terms of any desired measure of total nutritive energy by the inclusion of appropriate procedures. Most commonly the data obtained with farm animals are expressed as total digestible nutrients, either by including digestion trials or by the use of average coefficients of digestibility (Sec. 190).

The feeding-trial method of determining the energy requirements for growth is illustrated by the studies of Eckles and Gullickson<sup>1</sup> with Holstein and Jersey cattle, based upon data for 50 animals divided among three levels of energy feeding. The intakes of those which were considered to have made a normal growth, as measured by weight, were then averaged to provide the data selected to represent the requirements. These estimated requirements are given in Table XXIV.

TABLE XXIV.—TOTAL DIGESTIBLE NUTRIENTS IN POUNDS REQUIRED BY GROWING DAIRY CATTLE

Body weight	Total digestible nutrients	Body weight	Total digestible nutrients
100	2.32	450	6.77
150	3.36	500	7.06
200	4.23	550	7.34
250	4.96	600	7.65
300	5.55	650	7.96
350	6.03	700	8.26
400	6.44	750	8.56

Actually, the feeding trials in which any attempt has been made to determine the minimum intakes of energy required for

<sup>1</sup>ECKLES, C. H., and T. W. GULLICKSON, Nutrient requirements for normal growth of dairy cattle, *J. Agr. Research*, **42**, 603-616, 1931.

growth are very few. Most of them have involved ad libitum feeding, and the results are expressed in terms of feed required per 100 lb. gain. Undoubtedly these results do not represent the minimum requirements in many cases, particularly where unbalanced rations were used. The better conducted experiments do provide safe guides for practice which should be followed until more exact studies establish lower values which can be relied upon to be equally satisfactory. Any feeding standard for practice must allow a margin of safety for, if the values selected just meet the requirement determined as an average of the data obtained with a group of animals, half of them may be underfed with such an allowance. From a thorough study of feeding trials, Morrison<sup>1</sup> has set forth standards for the growth of all classes of farm stock.

**252. Coefficients for Computing Energy Requirements for Growth.**—The energy requirements of growing children are frequently computed by the use of coefficients which represent percentages of the requirement for maintenance of the adult. Since the time of Atwater, many different sets of coefficients have been proposed comprising different values for each year, corresponding to what is considered to be a normal rate of growth. For example, considering the requirement of the adult as 3000 Cal., The Bureau of Home Economics of the U. S. Department of Agriculture uses 0.4 as the coefficient for computing the requirements of children under four years, with increasing coefficients with age to maturity. These coefficients find an important use in computing the food requirements of a population, based upon the number of "adult-male units" represented and in computing diets for families as is illustrated in the bulletin by Stiebeling and Munsell<sup>2</sup>.

**253. Fat Content of the Growth Ration.**—Within certain limits, at least, the energy requirement for growth can be supplied by either carbohydrate or fat. In the usual rations, carbohydrate is by far the principal source. The only practical problem arising is that of the kind of fat, as in the production of soft pork. The

<sup>1</sup> MORRISON, F. B., Feeds and feeding, Morrison Publishing Company, Ithaca, N. Y.

<sup>2</sup> STIEBELING, HAZEL K., and HAZEL E. MUNSELL, Food supply and pellagra incidence in 73 South Carolina farm families, *U. S. Dept. Agr. Tech. Bull.* 333, 1932.

question as to whether a certain minimum fat intake is desirable has been discussed (Sec. 73). The evidence is mostly negative in so far as the growth of farm animals is concerned. A certain percentage of the calories as fat is recommended for human diets, but it is agreed that the basis of this recommendation remains uncertain.

#### THE PROTEIN REQUIREMENTS FOR GROWTH

Aside from water, the body increase during growth consists very largely of protein. The theoretical minimum protein requirement is the amount actually stored in the body. But this is far below the actual requirement because of the wastage in digestion and metabolism. The loss in digestion can be taken account of by stating the requirement in terms of digestible protein, and data on digestibility are available for all of the common feeds. The wastage in metabolism is much less readily assessed. It is governed primarily by the efficiency with which the digested protein supplies the amino acids required for the construction of body tissue, a measure which constitutes the biological value (Sec. 86) of the food protein. The wastage here concerned is a large and variable one; and a consideration of the data available on the biological value of different protein sources for growth is a logical starting point for a discussion of the protein requirement.

**254. The Determination of Biological Value.**—The protein efficiency or biological value of a given feed is measured as the percentage of the intake which is actually utilized in the body. It is sometimes expressed as the percentage of the total intake that is stored. Here losses in digestion as well as in metabolism are taken into account. More commonly the losses in digestion are eliminated from consideration by making the computation on the basis of the digested protein. This procedure measures the efficiency of the absorbed protein in supplying the amino acids needed for the synthesis of body protein, thus arriving at a figure for biological value which constitutes the more exact and preferred usage of the term. The calculation is made most simply as follows:

$$\frac{\text{N intake} - (\text{fecal N} + \text{urinary N}) \times 100}{\text{N intake} - \text{fecal N}} = \text{biological value}$$



It is evident that the data for such a calculation can be obtained from a nitrogen-balance experiment. The level of protein fed must be high enough so that growth will actually result as indicated by a positive balance; yet it must not be in excess of the amount needed to cause maximum growth, because an intake above this would be catabolized and excreted and thus give a biological value lower than the true one. There must be a sufficient intake of nonnitrogenous food so that the protein will not be needed as a source of energy.

**255. The Thomas-Mitchell Method.**—A refined but more complicated method of measuring biological value takes into account the metabolic (Sec. 92) and endogenous nitrogen (Sec. 97). This method was originated in 1909 by Karl Thomas<sup>1</sup> of Leipzig who first used the term, biological value. It is interesting to note that Thomas developed this concept and a method for its measurement prior to the work of Osborne and Mendel which inaugurated the modern studies of protein quality. The Thomas method has been modified and popularized by Mitchell<sup>2</sup> who has conducted a very large number of studies with it, and become a strong champion of its usefulness. The method is best explained by a consideration of the formula which Mitchell uses in calculating the value obtained:

$$\frac{\text{N intake} - (\text{fecal N} - \text{metabolic N}) - (\text{urinary N} - \text{endogenous N})}{\text{N intake} - (\text{fecal N} - \text{metabolic N})} \times 100$$

The feature of this formula, distinguishing it from the previous one, is its recognition of the fact that endogenous and metabolic nitrogen represent fractions which have actually been utilized by the body even though they appear as excretions. Thus, in the numerator, the fecal loss subtracted from the total intake is limited to the part actually undigested, and the urinary loss is

<sup>1</sup> THOMAS, KARL, Über die biologische Wertigkeit der stickstoff Substanzen in verschiedenen Nahrungsmittel, *Arch. Anat. Physiol.*, Physiol. Abt., 1909, pp. 219-302.

<sup>2</sup> MITCHELL, H. H., A method of determining the biological value of protein, *J. Biol. Chem.*, **58**, 873-903, 1924. MITCHELL, H. H., WISE BURGROUGHS, and JESSIE R. BEADLES, The significance and accuracy of biological values of proteins computed from nitrogen metabolism data, *J. Nutrition*, **11**, 257-274, 1936.

reduced by its endogenous fraction before being subtracted. The numerator, therefore, represents the total nitrogen utilized, including the part used in maintenance as well as that built into growth tissue. Since in the denominator also, the metabolic nitrogen is subtracted from the total fecal output, the biological value computed is the percentage of the actually digested nitrogen that is utilized. In excluding the metabolic and endogenous nitrogen from the losses it becomes a measure of the efficiency of the protein for the combined functions of growth and maintenance.

In principle the Thomas-Mitchell method is certainly an exact one for evaluating proteins, but the procedure used in carrying it out involves certain uncertainties and difficulties. The values for metabolic and endogenous nitrogen cannot be determined while the protein is under study, but must be calculated from values obtained in separate periods when the animals are receiving a nitrogen-free diet. Thus the method assumes that corresponding outputs occur during the protein test itself. As regards endogenous nitrogen, it thus assumes the constancy of output specified by the Folin theory (Sec. 97).

The data presented in Table XXV, taken from a publication by Mitchell,<sup>1</sup> illustrate the nature of the results obtained. These figures reveal the wide differences which exist in the efficiency of various proteins, as measured by the rat, and they illustrate the extensiveness of the role that biological value can play in governing the amount of dietary protein required.

TABLE XXV.—BIOLOGICAL VALUE OF THE PROTEINS OF HUMAN FOODS

Food	Biological value of protein, %	Food	Biological value of protein, %
Whole egg.....	94	Whole wheat.....	67
Milk.....	85	Potato.....	67
Egg white.....	83	Rolled oats.....	65
Beef liver.....	77	Whole corn.....	60
Beef heart.....	74	Wheat flour.....	52
Beef round.....	69	Navy beans (cooked)....	38

<sup>1</sup> MITCHELL, H. H., The protein values of foods in nutrition, *J. Home Econ.*, 19, 122-131, 1927.

The biological values given in Table XXV were determined by introducing the food in question into the basal diet in such amounts as would provide a protein level lying between 8 and 10 per cent. In addition to avoiding too high levels, it is necessary to hold to approximately the same level where the data obtained with different foods are to be compared. This is because biological value decreases with increase in protein level. This fact also indicates that determined values are more useful for comparative purposes than as absolute measures applicable to practice where different diets may vary markedly as to protein level.

**256. The Supplementary Relations among Proteins.**—Another reason why figures, such as those given in the preceding table, are more useful as relative measures than as absolute values to be used in selecting diets lies in the fact that when two protein sources are combined the resulting value is not necessarily the mean of the individual values. The explanation here is that certain proteins mutually supplement each other so that the resulting amino-acid mixture has a biological value superior to that of either protein when fed alone. This is strikingly illustrated by the studies of Mitchell and Kick<sup>1</sup> with corn and tankage, long known to represent an effective combination for the growth of swine. Using eight growing pigs the average biological values were: corn, 54; tankage, 42; the combination (2:1), 61. Though an animal product, tankage consists of tissues which by themselves furnish a very inefficient amino-acid mixture, but it supplies certain amino acids in which corn is deficient and the latter performs a similar service for tankage.

The different protein efficiencies which result when corn is combined with other feeds are illustrated by the data on page 338 taken from an extensive experiment by Hart and Steenbock<sup>2</sup> with growing pigs.

These data also show the differences in values obtained by two methods of calculating the efficiency. The percentage retention is always larger when computed on the basis of the absorbed

<sup>1</sup> MITCHELL, H. H., and C. H. KICK, The supplementary relation between the proteins of corn and of tankage determined by metabolism experiments on swine, *J. Agr. Research*, **35**, 857-864, 1927.

<sup>2</sup> HART, E. B., and H. STEENBOCK, Maintenance and production value of some protein mixtures, *J. Biol. Chem.*, **38**, 267-285, 1919.

nitrogen than on the basis of the total fed because of the loss in digestion. The lower the digestibility of the ration the greater the difference between the values calculated by the two methods.

Ration	Percentage of total nitrogen retained	Percentage of absorbed nitro- gen retained
Corn and alfalfa.....	31.7	47.3
Corn and tankage.....	40.3	56.7
Corn and milk.....	61.7	71.4

The experiments just cited also illustrate the fact that the most useful data for biological value are those obtained with specific combinations used in practice, since single feeds seldom provide the entire ration. Much useful information has thus been obtained as to the relative value of various protein supplements for swine feeding and, to a lesser extent, for other species. With rations which usually include several feeds and draw on a wide range of sources, such as those for dairy cattle and for man, studies of specific combinations have less application.

**257. Biological Values for Different Species.**—Most of the data for biological values have been obtained with rats. Several studies have been made with pigs and a few with sheep and cattle. The question as to the extent to which the values obtained with one species apply to another is an important one from the standpoint of their general usefulness, particularly in view of the special suitability of the rat for determining these values. There are several experiments, such as those of Schneider<sup>1</sup> with fish meals, indicating that the values obtained with rats tend to hold for pigs also. Chickens also appear to respond similarly to rats. Though the evidence is rather meager, the rat data have been frequently used in assessing human diets. For Herbivora, however, the situation is different, at least in certain respects.

The forages, which make up a large proportion of the rations of Herbivora, are not suitable for testing with the rat or other Omnivora because of limitations as to their digestibility. The biological value of the protein of the rations of Herbivora must

<sup>1</sup> SCHNEIDER, BURCH H., Nitrogen-balance studies with various fish meals, *J. Agr. Research*, 44, 723-732, 1932.

be determined with the Herbivora themselves. Very few carefully conducted studies have been made, and of these the majority have dealt with the more difficult question of biological value for milk production (Sec. 318) rather than for growth. The Cornell workers<sup>1</sup> have carried out a series of experiments with sheep, using the Thomas-Mitchell procedure for the most part. No differences were found between alfalfa and clover protein or between combinations of alfalfa and corn and clover and corn. When certain protein concentrates were fed as nearly the sole supply of nitrogen in a ration containing purified sources of other nutrients, the protein of soybean-oil meal was found superior to the protein of corn-gluten meal, as is shown below:

	Corn-gluten meal	Soybean-oil meal
Digestibility of nitrogen, per cent.....	66.3	67.0
Nitrogen intake stored, per cent.....	26.5	33.8
Digested nitrogen stored, per cent.....	39.8	51.0
Biological value (Thomas-Mitchell), per cent	65.7	72.8

The digestibility was the same for both feeds but the protein efficiency was superior for the soybean-oil meal, whether calculated on the basis of total nitrogen intake, the digested nitrogen, or as the biological value obtained by the Thomas-Mitchell procedure. These data also illustrate the different values obtained by the three methods of expressing protein efficiency. When in a later experiment soybean-oil meal and gluten feed were compared as supplements to timothy hay and corn, no differences were found.

Evidence from rat experiments that the proteins of leaf and seed tend to supplement each other is responsible for the suggestion that rations containing legume hay should have a higher biological value than those based upon grass hay. No evidence

<sup>1</sup> TURK, K. L., F. B. MORRISON, and L. A. MAYNARD, The nutritive value of the proteins of alfalfa hay and clover hay when fed alone and in combination with the proteins of corn, *J. Agr. Research*, **48**, 555-570, 1934; The nutritive value of the proteins of corn-gluten meal, linseed meal, and soybean-oil meal, *ibid.*, **51**, 401-412, 1935. MILLER, JOHN I., F. B. MORRISON, and L. A. MAYNARD, Relative efficiency for growing lambs of protein in rations supplemented by soybean-oil meal, linseed meal or corn-gluten meal, *J. Agr. Research*, **54**, 437-448, 1937.

for this viewpoint was obtained in an experiment by Maynard and associates with lactating cows (Sec. 319). In growth studies with rabbits, Crampton and associates<sup>1</sup> have produced evidence that the nutritive value of certain pasture grasses can be improved by the addition of a complete protein (casein).

It remains to be proved that any large variations in biological value exist among the combinations of feeds which make up the usual rations of Herbivora. In fact, large differences are unlikely in view of the nature of the rations used, for the studies which have been made of plant products with rats have revealed few cases showing large variations, in contrast to the results obtained with and without the inclusion of the better animal sources. It is also possible that the biological value of the protein as fed to Herbivora is of less importance than in Omnivora, because of the extensive bacterial action in the rumen whereby the amino-acid mixture presented for absorption may be different and more effective than that originally present in the feed (Sec. 90). This attractive explanation requires proof.

**258. Protein Efficiency as Measured by Feeding Trials.**—The efficiency of a given protein in causing the building of protein tissue in growth can be determined by a feeding trial in which the nitrogen retained is measured by slaughter and analysis of the animals. This procedure has been used in studies with rats and to a lesser extent with pigs. As a method of comparing the relative value of two or more foods or rations long-time feeding trials have been carried out in which the data have been limited to growth and feed records, and in which the comparison is made on the basis of the gain in weight per-unit of protein intake. While obviously a less exact measure than the nitrogen-balance or slaughter procedure, this method more closely duplicates the conditions of practice in that a better rate of growth may be obtained and when properly carried out gives results of definite value. McCollum and his associates<sup>2</sup> conducted a large number

<sup>1</sup> CRAMPTON, E. W., Pasture studies IV. Nutritive value of pasture herbage: quality of protein, *Empire J. Exptl. Agr.*, **2**, 337–348, 1934. CRAMPTON, E. W., and D. A. FINLAYSON, Pasture studies VII. The effect of fertilization on the nutritive value of pasture grass, *ibid.*, **3**, 331–345, 1935.

<sup>2</sup> MCCOLLUM, E. V., NINA SIMMONDS, and H. T. PARSONS, Supplementary protein values of foods: I. The nutritive properties of animal tissues, *J. Biol. Chem.*, **47**, 111–137, 1921. (See also several following papers in the same journal.)

of studies in which the protein sources to be compared were introduced into rations at a 9 per cent level and fed to rats through two or three generations, using the combined records of growth, reproduction, lactation, and longevity as measures of the relative value of the different sources. They used these results as a basis of classifying various foods as regards the efficiency of their proteins. Though the work has been criticized because of lack of data as to food intake and because other factors besides protein might come into play over a generation, from the standpoint of growth for productive life, the data provide information not given by those of any other workers.

**259. Comparative Biological Value of Various Foods.**—On the basis of the studies with rats and the much fewer ones which have been made with pigs and chicks, certain generalizations can be made regarding the comparative biological value of individual foods and combinations. Animal products as a class are superior to vegetable foods. At the top, stand milk and eggs, followed by liver, kidney, and other glandular organs. Muscle meat ranks somewhat lower but is above seeds and other vegetable products. Certain animal tissues, however, such as blood, connective tissue, and others, rank well below the seeds, and this fact explains the low value for tankage. Most of the whole seeds do not show wide differences from each other but navy beans, cow peas, and lentils are distinctly inferior. The cereal seeds have higher values than the flours made from them. Thus milling by-products tend to be higher than whole seeds or their flours. In general there seem to be no marked differences between the seed and the vegetative portion of a given plant.

Since these products are seldom used as the sole source of protein, the values for combinations of them are of more practical importance. Combinations of vegetable and animal products generally provide effective mixtures, and, when seeds are supplemented with 10 to 15 per cent of one of the better animal products, the combination is nearly as good as the animal source alone. In the case of corn and tankage, it is better. In general, seeds and their products do not supplement each other, but a combination of soybeans or peanuts with cereal seeds is an exception. For swine and chicks milk is a better supplement to cereal grains than is meat scraps or tankage. Soybean-oil meal has been found as good as the slaughter-house by-products and certain fish meals

have proven superior. There is no basis for making any statements regarding the combinations commonly used for Herbivora.

**260. Factors Governing the Protein Requirement.**—As commonly stated in feeding standards, the protein requirement for growth includes the amount needed for maintenance as well. The maintenance component increases with body size, but the demand per unit of new tissue formed decreases with age and body size because of the decreasing protein content of this tissue. While the total daily requirement increases with age and size, it decreases per unit of weight and in relation to the energy requirement. For example, according to the average values of the Morrison standard, a 50-lb. lamb requires 0.175 lb. of digestible protein daily in a ration having a nutritive ratio of 1:6.75, while a lamb weighing twice as much needs 0.26 lb. with a ratio of 1:7.75. Standards for children, expressed as grams per kilogram of body weight, decrease from 3.5 g. during the first year to 1 g. at maturity.

In view of the factor of biological value, it is evident that there can be no fixed minimum requirement except in terms of specific food sources. The fact that biological value decreases with level of intake has the effect of increasing the requirement per unit of tissue gained as the growth rate is increased. The previously discussed evidence (Sec. 218) that, within rather wide limits, the wastage of energy in metabolism is decreased as the level of protein is raised suggests that the most efficient level of intake may be higher than the amount needed for its specific function as protein. All of these considerations suggest higher allowances in practice than certain minimum values which have been determined for specific combinations.

**261. Estimation of Protein Requirement from Slaughter and Metabolism Data.**—The basic factor in the protein requirement is the amount built into growth tissue. This can be determined either by slaughter tests or by a nitrogen balance. Studies in which animals were killed and analyzed periodically during growth have provided rather complete data for various species as to the increase in protein at different stages. Mitchell<sup>1</sup> has used these data to arrive at what he refers to as the total net-protein requirement. It comprises the sum of the physiological maintenance

<sup>1</sup> MITCHELL, H. H., Relations between the metabolism of protein and of energy, *Proc. Am. Soc. Animal Production*, 1933, pp. 229-232.



value obtained from data on endogenous nitrogen plus the increase in protein tissue. A series of values for the daily intake are thus presented, corresponding to different stages of growth. Mitchell states that, in translating these data for net requirements into a digestible protein requirement for practice, a biological value must be assumed. He considers 50 per cent to be a fair value for this purpose. The requirements as thus arrived at are below those suggested by feeding trials. Mitchell put forth his values primarily to illustrate a physiological basis of arriving at the requirements, and he recognized that they need testing in practice.

The nitrogen-balance method can be used to measure the actual requirement in terms of a specific food or ration by determining the lowest level of intake which will give the maximum total retention. In view of the changes with age, the procedure must be carried out at various stages of the growth period in order to provide the data required. Mitchell and Hamilton<sup>1</sup> have made a brief report of an extensive experiment with pigs, carried out by the paired-feeding technique, in which the nitrogen retained was used as a measure of the protein requirement. On this basis a ration containing 15 per cent of protein proved superior to those containing lower levels, for pigs weighing from 130 to 186 lb. as well as for younger pigs. A ration supplying 18 per cent of protein caused a larger retention than one furnishing 15 per cent for pigs weighing from 42 to 70 lb., but not for those weighing 140 lb. or more. Digestibility data were obtained as were also growth records which checked the nitrogen-balance results. It was concluded that pigs weighing from 40 to 100 lb. require a ration containing more than 17 per cent of total protein, corresponding to 15.4 per cent of the digestible nutrient; but that, for higher weights, a total figure of 15 per cent, corresponding to 12 per cent digestible protein, is sufficient.

**262. Protein Requirement from Feeding Trials.**—In the feeding-trial method of measuring the protein requirement different levels are fed to find the minimum one which will give a maximum rate of growth. The inclusion of slaughter tests to show the nature of the increase made provides valuable additional data which are obtainable in the case of meat animals. The studies

<sup>1</sup> MITCHELL, H. H., and T. S. HAMILTON, The balancing of rations with respect to protein, *Proc. Am. Soc. Animal Production*, 1935, pp. 241-252.

with chicks by Norris and Heuser,<sup>1</sup> in which rations containing different percentages of protein were prepared by varying the amount of meat scrap included, illustrate the feeding-trial method. Levels varying from approximately 13 to 21 per cent were compared. The requirement was found to be higher than 18.58 per cent for the first eight weeks, whereas a level of 15 to 16 per cent was adequate thereafter. The birds were fed ad libitum, and the highest level of protein intake gave the largest gain per gram of feed during the first eight weeks, as well as the greatest total gain. The gain per gram of protein decreased with the level of intake as was to be expected.

The previously mentioned experiments of Ellis and Hankins<sup>2</sup> with pigs in which the protein levels were 12.4, 15.4, and 18.9 per cent furnish feeding-trial data which are in essential agreement with the requirements suggested by Mitchell and Hamilton from their slaughter studies (Sec. 261).

In 1917 a cooperative study of the protein requirements for the growth of cattle was undertaken under the auspices of the National Research Council, directed by Armsby who had worked out the detailed plan much earlier but met with no success in interesting experiment stations to cooperate. From 1918 to 1923 studies were carried out at seven different stations and the data obtained have been summarized in two bulletins of the council.<sup>3</sup> The plan, involving both growth and nitrogen-balance studies, was not fully carried out at any station and many modifications were made in its procedures. Thus success was not achieved in producing a large body of data which could be summarized as a unit to answer the question under investigation. At the close of the second bulletin a summary is presented of selected data, giving the minimum protein intakes of animals, six months of age and older at the start, which made gains considered to be normal over an experimental period ranging from 150 to 400 days for different individuals. These intakes varied from 1.17 to 1.69 lb.

<sup>1</sup> NORRIS, L. C., and G. F. HEUSER, The relation of the protein requirement of chicks to the rate of growth. I. The quantity of protein required by chicks during early growth, *Poultry Sci.*, **9**, 378-392, 1930.

<sup>2</sup> ELLIS and HANKINS, *loc. cit.*

<sup>3</sup> ARMSBY, H. P., Cooperative experiments upon the protein requirements for the growth of cattle, *Nat. Research Council Bull.* 12, 1921. FORBES, E. B., Cooperative experiments upon the protein requirements for the growth of cattle, *ibid.*, 42, 1924.

per 1000 lb. live weight. The data actually obtained from these cooperative experiments are of limited value, but the excellent plan of Armsby and the experience gained with it remain as very valuable guides for further studies.

In any experiment set up to study the protein requirement, provision cannot be made for keeping the biological value constant. Varying amounts of different sources of protein must be used, except where the purified-diet method is employed, and, even in the latter case, the values will vary with the level tested. This is not a serious limitation from the standpoint of arriving at reliable figures for practice, provided the feeds are properly selected. In the experiments previously cited, the shifts in the combinations of the ingredients were those that would normally be made in good feeding practice where a change in protein intake is desired. In the case of the studies with the pigs and chickens, the rations providing the minimum protein which gave the best results contained protein mixtures known to have good biological values for the species. The requirements suggested by these results should apply to the rations recommended to represent good feeding practice. They might prove inadequate for less efficient combinations.

**263. Methods of Stating the Protein Requirement.**—In the previously cited studies, the protein requirement has been expressed in different ways. It should be stated as digestible protein for those species for which digestibility data are available. Stating it as the pounds required per head per day for different body weights, as is done in the Morrison standards, provides an exact basis for modifying the intake in accordance with the decreasing requirement per unit of body weight. It should be recognized that such a statement cannot contain determined values for all weights but that many of the figures given must represent interpolations from weights at which determinations have actually been made.

Since the total food intake for growth is governed primarily by the energy needs, stating the protein requirement in relation to the energy intake has certain advantages. The nutritive ratio provides a means of doing this where digestibility data are available. A similar relationship is provided in dietary standards for man by the statement that the intake of protein calories should be 10 to 15 per cent of the total calories, using the factors employed

in calculating physiological fuel values (Sec. 203). Having specified the relation between energy and protein for a given age and selected the ration accordingly, the only intake figure needed is for the total food intake which will supply the energy requirement. This same procedure applies where the protein requirement is stated as a percentage of the total ration, but the latter statement, though simple, is a less exact method because digestibility is not taken into account. It is most suitable for stating the requirements of those species, such as pigs and chickens, which are normally fed rations of rather high digestibility and thus not susceptible to wide variations in this respect. In contrast, the method is not applicable in the case of the Herbivora, because of the large differences in digestibility of the feeds which may make up their rations. The method loses its simplicity, anyway, when two or more components of the ration, such as grain, hay, and silage, are fed separately.

**264. Wool Production.**—Since the wool fiber is practically pure protein, a substantial amount of food protein is required for its production. Thus the protein requirements for sheep are somewhat higher than for cattle at the same stage of maturity. A deficiency of this nutrient or of energy results in a lighter fleece of poorer quality. Since wool protein is rich in the amino acid, cystine, many experiments have been conducted to ascertain whether a deficiency of this amino acid is a limiting factor in wool growth and whether commonly fed rations can be improved by additions of sources of it. The more carefully conducted studies have generally given negative results. In extensive and critical experiments, du Toit and associates<sup>1</sup> showed that neither the addition of cystine nor of other forms of sulfur to a basal ration of corn, hay, and green feed was effective, as measured by increase in body weight, yield of fleece, or quality of fleece. The amount of cystine stored as wool was practically equal to the cystine intake. Considering that a 100 per cent efficiency in the metabolism of ingested cystine was very improbable, the investigators expressed the view that the amount of cystine produced as wool is not entirely dependent on the amount fed. This implies that

<sup>1</sup> DU TOIT, P. J., A. I. MALAN, J. W. GROENEWALD, and M. L. BOTHA, Studies in mineral metabolism. XXXII. The effect of different forms of sulphur in the diet upon the growth and wool production of sheep, *Onderstepoort J. Vet. Sci.*, 4, 229-239, 1935.

cystine can be synthesized from some other sulfur-containing dietary constituent besides cystine itself. This idea is supported by the recent studies of Rose and associates (Sec. 85) showing that cystine is a nonessential amino acid for the growth of rats. In the ruminant a further possible explanation lies in the synthetic powers of rumen bacteria (Sec. 90).

#### CALCIUM, PHOSPHORUS, AND VITAMIN D

The general functions of the mineral elements and vitamins required by the body have been discussed in Chaps. VII and VIII. As a result of many studies, fairly reliable data for several species are available on the quantitative requirements for growth of those minerals which particularly need attention to insure that the ration selected will contain an adequate supply. Much fewer quantitative data are at hand regarding the requirements for the vitamins because the knowledge of this field is so recent and so incomplete. The calcium, phosphorus, and vitamin D needs for growth have been extensively studied in farm animals, not only because of their individual importance but also because of their interrelations in metabolism (Sec. 103). They are best discussed together.

Vitamin D is essential for the growth of all species of higher animals in so far as studied, but there are marked species differences as regards quantitative needs. There are also species differences with respect to the requirements for calcium and phosphorus, depending upon the rate of growth and other factors. In every case the interrelations between the three nutrients are such that a minimum requirement for a given one cannot be specified except in terms of rather definite amounts of the other two also. Failure to recognize this fact in many of the experiments, designed to determine requirements, limits the usefulness of the data obtained. Since the intakes in diets consisting of natural feeds cannot, at best, be adjusted exactly to the optimum relations which make possible a minimum requirement, recommendations in practice must specify more liberal allowances accordingly.

By far the principal needs for calcium, phosphorus, and vitamin D during growth are for the formation of the skeleton and thus they may be termed the bone-forming nutrients. Phosphorus plays an important role also in the growth of the soft tissues, as is

evident from its occurrence in important amounts elsewhere in the body than in the bones (Sec. 105). In addition, this element exerts an indirect effect on growth because of its relation to appetite. To a lesser extent this is true also for calcium and vitamin D.

**265. Measures of Skeletal Growth.**—The development of the skeleton cannot be measured by increase in weight, nor can its adequacy be determined by dimensional measures of the body or even of the bones themselves. Size of bone is governed largely by inheritance and a large bone may be a very weak one if the nutrition has not been adequate. Severe nutritive deficiencies during growth manifest themselves in misshapen bones as in severe rickets (Sec. 109), but mild deficiencies may have serious consequences without any evident early symptoms. There may be later deformities or fractures or a breakdown of the teeth, as a result of prolonged periods of mild deficiency. Even though these evident symptoms never occur, the bone development may still be inadequate, particularly as regards its content of calcium and phosphorus reserves which are normally called upon during reproduction and especially during lactation (Sec. 325).

The real measure of the adequacy of skeletal development is the density and strength of the bones formed as conditioned by their content of calcium and phosphorus and their histological structure. Thus the requirements for the bone-forming nutrients can be determined by slaughter experiments in which representative bones are analyzed for calcium and phosphorus or studied histologically. The measurement of density and hardness and the determination of breaking strength are useful supplementary measures. Since the ash of bone consists almost entirely of calcium and phosphorus and since this remains true no matter what the quality of the bones, the determination of the ash is more commonly used as the measure of the adequacy of bone nutrition than the more time-consuming analyses for calcium and phosphorus. The progress of bone development can be followed quantitatively in the living animal by calcium and phosphorus balances. Useful supplementary measures of the state of the nutrition of both elements are the level of the inorganic phosphorus in the blood and X-ray photographs.

**266. Optimum Bone Development.**—In the discussion of bone growth in Chap. VII, it was pointed out that calcium and phos-

phorus are deposited in the bone as reserve material as well as constituents of the structural portion itself. Presumably the building of the latter has first call on the bone-forming nutrients, but where the intakes are large enough deposition in the trabeculae doubtless occurs also. In the case of animals grown for slaughter, the state of the reserves would seem to be of minor importance, but other considerations enter for those being reared for breeding and milk production.

In an interesting study of skeletal development in the rat, Outhouse and Mendel<sup>1</sup> found that an increased rate of body growth, caused by a diet richer in various nutritive factors including calcium, was accompanied by an increased rate of calcification compared to that in the slower growing animals. When the latter reached the same mature body weight, however, there were no differences between the two groups as regards the ash content of the bones. Thus it is suggested that, if bone development keeps pace with body growth, the end result is satisfactory, even though the rate of calcification is not at its maximum. Such is not the case if the calcification lags markedly behind body growth. Sherman and Booher<sup>2</sup> have reported that as diets containing increasing levels of calcium are employed, not only is maturity reached earlier but an increased storage of calcium per unit of body weight also results. They consider that the diet which causes the maximum rate of retention should be considered optimum until the normal store at maturity, including reserves, is attained. There are special reasons for believing that this is true in the case of animals which are to become milking cows, and in fact for all breeding females, because of the desirability of providing them with liberal reserves before they are subjected to gestation and lactation. This must be accomplished by a ration which will cause a rapid rate of calcification throughout the growth period, or else the time of breeding must be delayed accordingly.

While, within rather wide limits, increasing the bone-building nutrients in the ration results in an increased storage in the bones,

<sup>1</sup> OUTHOUSE, JULIA, and LAFAYETTE B. MENDEL, The rate of growth. I. Its influence on the skeletal development of the albino rat, *J. Exptl. Zool.*, **64**, 257-285, 1933.

<sup>2</sup> SHERMAN, H. C. and L. E. BOOHER, The calcium content of the body in relation to that of the food, *J. Biol. Chem.*, **93**, 93-103, 1931.

the percentage retention falls off markedly at the higher intake levels. This suggests that, as the limit of the capacity for the deposition of reserves is approached, the process becomes increasingly less efficient. If a maximum rate of calcification is considered to be optimum, a high intake in proportion to the amount stored must be supplied. There is no present reason to believe that calcification can be overdone during growth, except by massive doses of vitamin D (Sec. 157), nor is there evidence that excretory or other functions are unduly burdened by the ingestion of amounts of calcium and phosphorus which are large compared to the amounts stored.

**267. Calcium and Phosphorus Requirements Measured by Balance Studies.**—The generally recommended intakes of calcium and phosphorus for children are based upon balance studies by Sherman and Hawley,<sup>1</sup> involving periodic determinations on 21 individuals varying in age from three to thirteen years. The amounts of calcium stored were markedly greater when 1000 g. of milk were fed daily than when the intake was 750 g., from which it was concluded that children should receive an average daily intake of at least a gram of calcium to provide maximum storage. A somewhat larger intake of phosphorus was indicated. These results provided the basis of Sherman's recommendation that the child should receive a quart of milk a day to insure optimum intakes of the bone-forming minerals. His experiments were made before the importance of vitamin D was understood, but he has later pointed out that the children under study were in the sunshine much of the time before and during the experiments and thus that the vitamin was actually supplied.

Archibald and Bennett<sup>2</sup> have used the balance method in studying the phosphorus requirements of dairy heifers. Some of their data, representing summarized values for the trials noted, are presented in Table XXVI.

It is noted that the amounts of phosphorus retained were larger for the high-phosphorus ration for each group. The difference is nearly 40 per cent for the calves, a large figure which was shown

<sup>1</sup> SHERMAN, H. C., and EDITH HAWLEY, Calcium and phosphorus metabolism in childhood, *J. Biol. Chem.*, **53**, 375-399, 1922.

<sup>2</sup> ARCHIBALD, J. G., and E. BENNETT, The phosphorus requirements of dairy heifers, *J. Agr. Research*, **51**, 83-96, 1935.



statistically to be clearly significant. It is thus demonstrated that calves under 1 year of age require considerably more than 1.8 g. of phosphorus daily per 100 lb. live weight. Whether the intake should be as high as 3.25 g. or even higher cannot be stated without further comparisons. While not statistically significant, the differences shown by the amounts retained as yearlings and two-year-olds suggest that the intakes on the low-phosphorus ration were inadequate for maximum retention for these ages also. The data for percentage retained reveal the expected lower efficiency of higher intakes which occurs even though the total retention is greater. In the one case where the intakes were identical (high-phosphorus group, yearlings and two-year-olds) the declining rate of retention with age is revealed.

TABLE XXVI.—PHOSPHORUS BALANCES WITH DAIRY HEIFERS

Ration and period	Daily P intake per 100 lb. live weight, g.	Daily P retained per 100 lb. live weight, g.	Percentage of intake retained, %
High-phosphorus ration:			
As calves, 16 trials.....	3.25	1.01	31.08
As yearlings, 21 trials.....	2.49	0.61	24.50
As 2-year-olds, 5 trials.....	2.49	0.37	14.86
Average (weighted).....	2.78	0.73	26.26
Low-phosphorus ration:			
As calves, 17 trials.....	1.80	0.73	40.56
As yearlings, 13 trials.....	1.68	0.54	32.14
As 2-year-olds, 5 trials.....	1.18	0.32	27.12
Average (weighted).....	1.67	0.60	35.93

**268. Calcium and Phosphorus Requirements from Growth and Bone Data.**—Aubel, Hughes, and Lienhardt<sup>1</sup> have compared rations containing various levels of phosphorus for pigs receiving ample D, using growth, blood, and bone analyses and breaking strength as measures. They have concluded that the level should be between 0.27 and 0.3 per cent of the total food intake for the growth period as a whole. This percentage level corresponds to a

<sup>1</sup> AUBEL, C. E., J. S. HUGHES and H. F. LIENHARDT, Phosphorus requirements in the rations of growing pigs, *Kans. Agr. Expt. Sta. Tech. Bull.* 41, 1936.

daily intake of 6.5 g. per 100 lb. live weight for 50-lb. pigs and 4 g. per 100 lb. for 200-lb. pigs. The data obtained for bone composition are illustrated by the following figures from one period of their first experiment:

Constituent	Lot I	Lot II	Lot III
Calcium in feed, per cent.....	0.77	0.78	0.77
Phosphorus in feed, per cent.....	0.18	0.33	0.59
Ash in femur and humerus, per cent.....	48.14	57.35	59.64
Calcium in femur and humerus, per cent.....	18.35	21.93	22.70
Phosphorus in femur and humerus, per cent.....	8.69	10.58	10.82

It is noted that the calcium was held constant in all lots at a level which was certainly adequate, while the phosphorus was varied. Such a scheme unavoidably involves a variation in the ratio between the two elements. The ratio of 4.4:1 existing for the ration fed Lot I may have been in part responsible for the poorer bone development, but the ample amount of vitamin D supplied in each ration would tend to overcome any such effect. The data for ash, calcium, and phosphorus clearly reveal the superiority of the intermediate level over the lower one. While the data for the highest level are still better, the differences are small. For the experiment as a whole, the bone analyses and other measures employed failed to show significant differences in favor of the highest phosphorus level.

These data of Aubel and coworkers do not reveal the varying requirement with advance in age. The level recommended for the growth period as a whole may be inadequate for maximum calcification at the start and unnecessarily high at the end, but, since no higher level produced bones which were superior at the end, the level recommended is certainly adequate for growing hogs for market.

**269. Calcium and Phosphorus Requirements as Measured by Blood Data.**—Inadequate bone nutrition is reflected in a lowering of the blood level of calcium or phosphorus or both. The blood picture varies according to the specific deficiency concerned and also according to the species. A rapid rate of body growth which is accompanied by normal levels of calcium and phosphorus in the blood is highly indicative of adequate skeletal development, and

these measures are frequently employed to determine the actual requirements for the minerals.

Huffman and associates<sup>1</sup> have used this procedure for studying the phosphorus requirements of dairy cows, resulting in the following conclusions as regards growth requirements. A ration containing 0.2 per cent of phosphorus caused a lowering of the blood phosphorus of calves, which persisted up to eighteen months of age. Intakes of 5.7 to 9.9 g. of phosphorus per day were inadequate where the calcium-phosphorus ratio was 4:1 or wider. An intake of 10.3 g. daily sufficed from three to six months of age. Ten to 12 g. daily were adequate from eighteen months to first calving. Similar studies by these Michigan workers have led to the conclusion that an intake of 6 to 12 g. of calcium daily from birth to two years of age is sufficient for the growth of calves.

Martin and Peirce<sup>2</sup> have studied the phosphorus requirements of lambs by blood analyses. They have reported that a ration supplying 0.6 g. of this element per day was inadequate for normal body growth and for the maintenance of the normal inorganic-phosphorus level in the blood, whereas an intake of 1.6 g. was satisfactory. The animals received cod-liver oil as a source of vitamin D. The calcium intake was 2.7 g. per day.

**270. Calcium and Phosphorus Requirements as a Percentage of the Total Ration.**—The number of experiments which have dealt with the question of the calcium and phosphorus requirements for growth of the different species by one or another of the methods previously described are large indeed. But the data as a whole are incomplete and in many cases so contradictory that it is very hazardous to draw conclusions from them as to what the actual requirements are for a given species over its entire growth period. It is agreed that the requirements decrease with age per unit of body weight and also per unit of dry-matter intake. The requirements may be conveniently expressed as

<sup>1</sup> HUFFMAN, C. F., C. W. DUNCAN, C. S. ROBINSON, and L. W. LAMB, Phosphorus requirement of dairy cattle when alfalfa furnishes the principal source of protein, *Mich. Agr. Expt. Sta. Tech. Bull.* 134, 1933.

<sup>2</sup> MARTIN, CHARLES D., and A. W. PEIRCE, Studies in the phosphorus requirements of sheep. I. The effect on young Merino sheep of a diet deficient in phosphorus but containing digestible protein and vitamins, *Australia Council Sci. Ind. Bull.* 77, 1934.

percentages of the dry matter of the ration, from which the intakes per day can be calculated by a consideration of the amounts of dry matter fed at different ages.

Without any intention of suggesting a feeding standard, the following figures are proposed as intakes which should be adequate, based on a study of what appear to be the more reliable of the many experiments conducted. The figures are given as a range, the highest representing the requirement at weaning (or hatching) and the lowest the value to which the percentage may gradually fall during the course of growth. The average for a given range would not be optimum for the first part of growth, but continuing the maximum intake proposed throughout the growth period is clearly unnecessary.

Species	Calcium in dry matter, per cent	Phosphorus in dry matter, per cent
Chicken.....	1.0 to 0.5	0.7 to 0.4
Pig.....	0.6 to 0.30	0.40 to 0.25
Calf.....	0.45 to 0.20	0.38 to 0.20
Lamb.....	0.25 to 0.2	0.22 to 0.18

These data assume that vitamin D is supplied in accordance with the needs of the species in question. It should be remembered that the figures would be approximately 10 per cent lower on the basis of the air-dry ration. The percentages proposed for lambs rest on very fragmentary evidence. There is still less information available for colts, but their requirements are evidently closer to those of lambs than of cattle.

**271. The Requirements for Vitamin D.**—Methods of measuring and expressing the quantitative needs of the body for vitamins represent a very recent development, and data as to the amounts present in various feeds accumulate only slowly because biological methods of assay must be relied upon to a large extent. Thus recommendations for meeting growth needs do not, for the most part, rest on quantitative data as to body requirements and content in feeds, but rather consist of specifications as to what foods should be included to insure an adequate supply of a given vitamin. Of all species of farm animals, the chicken has the

largest requirement for vitamin D, and it is for this species that the needs have been most exactly determined.

The recent studies of Murphy, Hunter, and Knandel<sup>1</sup> with growing chicks and laying hens may be cited as an example of the procedure and measures which are involved in a quantitative determination of the vitamin D requirement. In their experiments with growing chicks, a diet containing adequate amounts of all other nutrients including calcium and phosphorus, but entirely lacking in vitamin D was employed. One group of chickens was placed on this diet while others were placed on the same diet plus a cod-liver-oil concentrate added in amounts which furnished graded levels of the vitamin for the different groups. In addition to using growth and feed consumption as measures, animals from each group were killed at eight, sixteen, and twenty-four weeks for bone studies which consisted of ash analyses and examination of longitudinal sections. Determinations were made also of the calcium in the blood serum. As an illustration of the data obtained some of the results of one experiment are given in Table XXVII.

The chicks receiving the basal ration alone showed external symptoms of rickets within three and one-half weeks. The data in the table reveal the very poor results obtained with this ration compared to where it was supplemented with the vitamin. The addition of 10 units was clearly insufficient, and the suggestive evidence that 19 units did not give optimum results in all cases was confirmed by the examination of the bone sections and by other experiments. The investigators concluded that 19 U.S.P. units of vitamin D per 100 g. of feed is the minimum protective level for chicks deprived of sunshine from hatching to twenty-four weeks of age, and that 39 units is an effective and economical addendum for the most adverse conditions. Their studies also showed that no addition was required where the chicks were reared in sunshine.

In connection with any statement of the vitamin D requirements of chicks expressed in rat units, it should be remembered that all sources of the vitamin are not equally effective per rat unit when fed to poultry (Sec. 152). For example, a larger

<sup>1</sup> MURPHY, R. R., J. E. HUNTER, and H. C. KNADEL, The vitamin D requirements of growing chicks and laying hens, *Pa. Agr. Expt. Sta. Bull.* 334, 1936.

number of units as irradiated ergosterol would be required than as cod-liver oil. For this reason the requirements of poultry are frequently expressed directly in chick units.

TABLE XXVII.—THE VITAMIN D REQUIREMENTS OF CHICKS

Weeks on experiment	Measures of adequacy	U.S.P. units vitamin D per 100 g. feed				
		0	10	19	39	58
8	Body weight, lb.....	0.66	0.98	1.19	1.23	1.19
	Ash in tibiae, per cent.....	39.2	44.6	51.1	51.1	51.2
	Ca in serum, mg. per 100 cc....	4.8	7.5	9.4	11.4	10.3
16	Body weight, lb.....	1.34	1.92	2.49	2.50	2.59
	Ash in tibiae, per cent.....	47.1	50.9	51.0	52.9	52.5
	Ca in serum, mg. per 100 cc....	5.6	8.6	9.5	10.3	10.6
24	Body weight, lb.....	*	2.57	3.35	3.40	3.37
	Ash in tibiae, per cent.....	*	49.2	56.5	56.4	54.5
	Ca in serum, mg. per 100 cc....	*	10.4	11.6	11.8	11.9

\* Killed after sixteen weeks.

Several experiments have shown that dairy calves develop the external symptoms of rickets accompanied by bone and blood changes when they are fed an otherwise adequate ration lacking in vitamin D and that the symptoms can be prevented or cured by cod-liver oil, irradiated ergosterol, and other antirachitic agents (Sec. 109). For the most part the potency of the agent used was not stated, and thus the requirements cannot be interpreted in terms of units. Long, Huffman, and Duncan<sup>1</sup> have reported a study of the vitamin D requirements of calves fed a ration adequate in calcium and phosphorus but lacking in the vitamin other than that supplied by the milk fed. They state that the need for the vitamin is approximately proportional to body weight and that, when winter or early spring whole milk is fed, the additional daily requirement of the vitamin is 0.3 to 0.4 U.S.P. units per pound of body weight. The blood-calcium level and bone studies were used as the measures. In an earlier study these Michigan

<sup>1</sup> LONG, JOHN W., C. F. HUFFMAN, and C. W. DUNCAN, A study of the vitamin D requirements of calves when natural milk furnished the sole source of the antirachitic factor, *Milk Plant Monthly*, 25 (7), 30-36; 1936.

workers found that 2 lb. daily of sun-cured alfalfa or timothy hay were sufficient to protect young calves against rickets but that older calves needed larger amounts. Summer sunlight is an effective antirachitic agent for calves.

Experiments have shown that rickets in pigs and in lambs can be prevented and cured by various dietary sources of vitamin D as well as by sunlight, but no quantitative data as to the requirements expressed in units are available. It is very evident that when there is a liberal intake of calcium and phosphorus in the proper ratio the need for the pig is less than for the chick or calf. Turkeys and pheasants have higher requirements than chicks.

**272. Meeting the Requirements for Bone-forming Nutrients in Practice.**—Nursing young never suffer from a deficiency of calcium or phosphorus and neither does the dairy calf reared on a liberal supply of skim milk. Herbivorous animals will receive ample calcium if their roughage is one-half or more legume hay, but where grass hay is the sole roughage and particularly where it is not consumed liberally a calcium supplement should be included unless the hay is known to contain 0.5 per cent or more of this element. No concentrate mixture is rich in calcium, but a liberally fed mixture which contains 25 per cent or more of some phosphorus-rich feed, such as wheat bran or one of the oil meals, will take care of the needs for the latter element, unless the roughage is unusually low in it. Roughage alone will not suffice. Under some conditions of practice, it is more economical to make up the deficiency in the roughage and cereal-grain ration by adding bone meal rather than a high-phosphorus concentrate, as has been shown by the experiments of Maynard and associates.<sup>1</sup>

Whether or not herbivorous animals on pasture will receive enough bone-forming minerals depends upon the fertility of the soil and the resulting calcium and phosphorus content of the forage. In the absence of specific information in these respects, giving the grazing animals access to bone meal is a desirable procedure. These various provisions for insuring adequate minerals in the rations of Herbivora require more attention in the case of calves than in the case of lambs and colts, because of the lower requirements of the latter. For a given species, the

<sup>1</sup> MAYNARD, E. J., J. E. GREAVES, and H. H. SMITH, Phosphorus supplements improve sugar-beet by-product rations for cattle, *Utah Agr. Expt. Sta. Bull.* 265, 1936.

provisions demand somewhat less attention with advancing age because of the decreasing requirements.

If pigs are being fed corn or other cereal grain with the additional protein required furnished as tankage or fish meal, they will receive sufficient calcium and phosphorus. Where a vegetable-protein concentrate is used instead, additional calcium is required, and it can be supplied as a mineral supplement. Where a milk by-product is the protein concentrate, it must be liberally fed to meet the calcium requirements.

Animals which are outdoors in summer will always receive plenty of vitamin D, but, in winter, the sunlight is an uncertain source in the northern latitudes even though the weather allows the animals to be outside much of the time, because of the generally less sunlight and its lower efficiency (Sec. 156). Whole milk cannot be relied upon to supply the vitamin D requirements of the calf and skim milk is certainly deficient. The most common symptoms of rickets in calves are swollen hocks and a tendency of the animal to stand with the back humped, but absence of these physical symptoms does not guarantee optimum bone nutrition. Sun-cured hay liberally fed will provide an adequate amount of the vitamin. When such hay is not available a vitamin supplement should be fed to animals not regularly exposed to summer sunlight.

The vitamin D requirements of pigs are evidently low where the ration contains an ample supply of calcium and phosphorus. Including 5 per cent of chopped, sun-cured alfalfa in their winter ration will usually meet their needs. From general considerations it would seem desirable to provide lambs with sun-cured roughage when they are fed and housed inside, but it cannot be said that there is any specific knowledge of their requirements for vitamin D, although they have been found to respond to it under certain conditions.<sup>1</sup>

Several carefully conducted experiments have shown that calves and pigs will receive no benefit from an addition of cod-liver oil or other special source of vitamin D when their rations are selected in accordance with the practices previously suggested. In general, it is preferable feeding practice to supply the

<sup>1</sup> AUCHINACHIE, D. W., and A. H. H. FRASER, The effect of lime and cod-liver oil on sheep fed on a calcium deficient ration, *J. Agr. Sci.*, **22**, 560-575, 1932.



mineral and vitamin needs of farm animals by an appropriate selection of the natural feeds rather than by resorting to special supplements.

#### OTHER MINERALS

**273. Iron.**—There are no quantitative data as to the iron requirements for the growth of farm animals, but several studies with children indicate that an intake of approximately 0.6 mg. per kilogram is adequate when a highly available source is employed (Sec. 123). An intake of this magnitude and more is supplied by the usual rations of farm animals, and there appears to be no practical problem as regards their iron requirements except in very special situations. The trace of copper that is needed for iron assimilation is also abundantly supplied by the commonly fed rations.

While a deficiency of iron in the soil and thus in the herbage has been reported from two or three areas in the world, the only common case of iron deficiency is that represented by the anemia which frequently occurs in suckling pigs. Owing to the labored breathing which is always characteristic of severe cases, this trouble was known as *thumps* long before it was discovered to be due to a lack of sufficient iron for blood formation. The trouble is most frequent in litters farrowed in late fall or early spring and kept inside without access to soil or forage. Anemic pigs are listless and flabby, their skin becomes wrinkled and their coats have an unhealthy appearance. As the disease progresses, the skin and mucous membranes become pale, and the animals become thin and weak. In advanced stages, the breathing is labored, and the pigs may have a swollen appearance, especially around the head and shoulders. This anemia can be prevented, or cured in its early stages by drenching the sucklings with a saturated solution of ferrous sulfate or other soluble iron salt. The weekly dosage is  $\frac{1}{3}$  teaspoonful for pigs under one week of age, up to 1 teaspoonful at four weeks. As an alternative procedure the iron solution can be swabbed daily on the udder of the sow. Whichever method is employed the commercial iron salt used will contain enough copper as an impurity to meet the need for this element.

This nutritional anemia in pigs and the recognized deficiency of iron and copper in the milk of all species have led to the suggestion

that all mammals should have supplements of these minerals during the suckling period. Such is not the case. While this anemia has been produced experimentally in calves and lambs, it does not occur in practice because these species begin to supplement their milk diet with other foods relatively earlier than does the pig. The store of iron with which they are born suffices until their needs are met adequately by grain and hay.

**274. Other Minerals.**—While it is recognized as desirable to add salt to the rations of all farm animals, very little information is available as to the exact requirements for its component minerals. South African workers have concluded that for growing cattle the daily requirements are only 1.5 g. of sodium and 5 g. of chlorine. The practice of allowing salt ad libitum to farm stock undoubtedly results in an intake in excess of requirements. There is no harm in this ordinarily, but certain species may be injured by overeating when salt is offered ad libitum after a long period of deprivation. Since salt increases the palatability of rations an intake in excess of the minimum physiological requirements is desirable from this standpoint. When it is fed free choice, the nature of the ration is a large factor governing the intake, and this may be in part a response to a variation in physiological need according to the mineral and other relations in the ration fed.

There are practically no specific data as to the iodine requirements for the growth of farm animals. Previous discussion has indicated that there is little if any definite evidence that commonly fed rations will be benefited by an addition of this element (Sec. 126). The recent studies of Malan and associates<sup>1</sup> showing that growing lambs may be harmed by low intakes of supplementary iodine have caused them to conclude that the use of such a supplement is to be discouraged unless a known deficiency exists. Other experiments testify to the correctness of this viewpoint.

Little, if anything, is known about the requirements of farm animals for the several other inorganic elements which are recognized as essential for growth on the basis of experiments with laboratory animals. The presumptive evidence that com-

<sup>1</sup> MALAN, A. I., P. J. DU TOIT, and J. W. GROENEWALD, Studies in mineral metabolism. XXXIII. Iodine in the nutrition of sheep, *Onderstepoort J. Vet. Sci.*, 5, 189-200, 1935.

monly fed rations are seldom if ever deficient in them has been discussed in Chap. VII.

#### VITAMIN A

All higher animals, in so far as they have been studied, require vitamin A for growth, and there is a considerable amount of information as to their quantitative requirements. In addition to the failure of growth there are various other characteristic deficiency symptoms which differ somewhat with the species (Sec. 142).

**275. Vitamin Requirement of Different Species.**—The need of vitamin A by calves was first shown by Jones, Eckles, and Palmer in 1926. Later studies have added greatly to the knowledge of the specific physiological effects and of the requirements. Guilbert and Hart<sup>1</sup> have given a very complete description of the various stages of vitamin A deficiency in calves and have determined the amounts of various sources of the vitamin which are required for prevention or cure. They found that the daily minimum requirement of carotene to prevent or cure clinical symptoms and to cause normal growth was from 26 to 33 micrograms per kilogram live weight. The same level of carotene was effective when fed as alfalfa, and a comparable vitamin A dosage was required as cod-liver oil or halibut-liver oil. The studies indicated that, in animals varying in weight from 130 to 500 kg., the requirement remained proportional to live weight and thus that it could be accurately stated on this basis. The effects of vitamin A deficiency in the calf are strikingly shown in Fig. 28.

Converse and Meigs<sup>2</sup> have described the occurrence of vitamin A troubles in calves reared on milk and skim milk from cows which had been fed for a considerable period on rations very low in the vitamin. The calves received whole milk for 20 days and then were gradually changed to skim milk, the change being completed at 30 days. They received supplementary grain and hay very low in the vitamin. When the milk and skim milk came from cows receiving timothy hay graded as No. 3, the calves died

<sup>1</sup> GUILBERT, H. R., and G. H. HART, Minimum vitamin A requirements with particular reference to cattle, *J. Nutrition*, 10, 409-427, 1935.

<sup>2</sup> CONVERSE, H. T., and E. B. MEIGS, Carotene and vitamin A in the nutrition of the dairy calf, *J. Dairy Sci.*, 17, 470, 1934, a paper read at the meeting of the Am. Dairy Sci. Assn., Ithaca, N. Y., 1934.

in from 45 to 89 days with the characteristic symptoms of deficiency. Milk produced from cows which received better hay resulted in a longer survival period, and pasture milk gave entirely satisfactory results in certain cases. The investigators concluded that the best whole milk, when fed for only 20 days, cannot be relied upon to provide enough storage of the vitamin in the calf to allow later satisfactory growth on skim milk unless hay of the proper quality or a supplement of the vitamin is fed. They suggest a daily allowance of 15 mg. of carotene. This is markedly

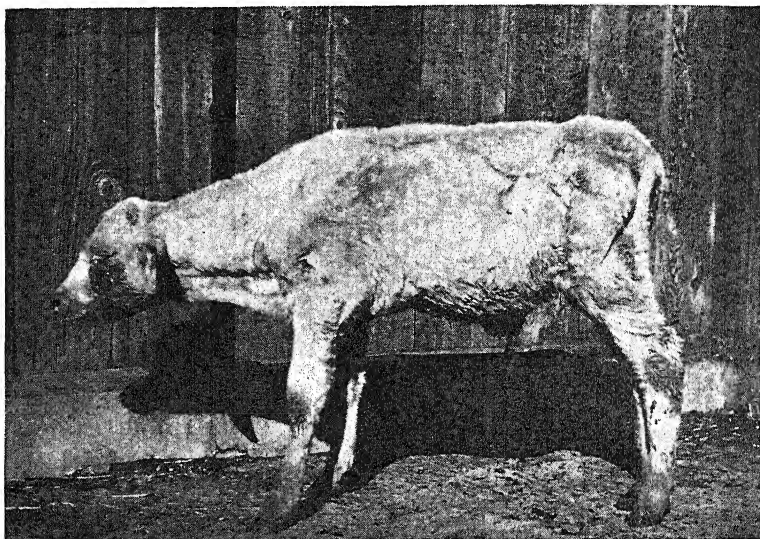


FIG. 28.—Three-month-old steer in moribund condition from vitamin-A deficiency. (Courtesy of G. H. Hart, University of California.)

in excess of the requirement for the young calf according to the figure of Guilbert and Hart, but it corresponds with their figure for an animal weighing 500 kg. The California workers agree that their minimum intake would not cause optimum storage.

The vitamin requirement of the growing calf can be amply provided by feeding green, leafy roughage. Properly cured alfalfa fed in as small amounts as 0.5 to 2 lb. daily, depending upon the size of the calf, will meet the requirement set up by the California studies. Including yellow corn in the grain mixture would help supply the need. The vitamin is furnished abundantly when the animals are on good pasture, but dried up grass

is very deficient, and animals grazed on it for extended periods develop marked symptoms of injury.

The need of pigs for vitamin A was clearly indicated by the early studies at the Wisconsin Experiment Station by Morrison, Bohstedt, and Fargo who showed yellow corn and tankage to be much superior to white corn and tankage for growth. Several later experiments<sup>1</sup> have provided information regarding the specific effects of its deficiency. Dunlop<sup>2</sup> found that an intake of 14 mg. of carotene per 100 lb. of feed provided nearly normal growth but that deficiency symptoms persisted. Sixty-two milligrams proved adequate for growth and provided some storage. Dunlop considers this as the minimum requirement and suggests that it be increased by 50 per cent as an allowance for practice. This allowance corresponds to a daily intake of 4 mg. of carotene for a 100-lb. pig. In their report on calves, Guilbert and Hart refer to unpublished data from their institution indicating that the requirement of the pig and sheep is of the same order as that of the calf. This would mean a lower intake for the pig than the minimum requirement suggested by Dunlop.

When the pig's ration consists principally of yellow corn the vitamin A requirement is probably taken care of adequately. The only cause for uncertainty here is the fact that this grain varies in vitamin value, and some samples have given very low figures. No other grain or by-product commonly fed to pigs will supply its needs. The inclusion of 5 per cent of green, leafy alfalfa, a practice which is the simplest way of meeting the needs for vitamin D also in the winter ration, will supply vitamin A adequately. Fresh, leafy forage crops supply the factor in abundance.

Several recent studies have indicated that the minimum vitamin requirement for the growth of chicks is from 50 to 100 micrograms of carotene daily or from 100 to 200 U.S.P. units of the vitamin per 100 g. of feed. A larger intake is required as a curative dosage than for prevention. Larger intakes than those

<sup>1</sup> HUGHES, J. S., C. E. AUBEL, and H. F. LIENHARDT. The importance of vitamin A and vitamin C in the ration of swine, *Kan. Agr. Expt. Sta. Tech. Bull.* 23, 1928.

<sup>2</sup> DUNLOP, GEORGE, Paralysis and avitaminosis A in swine, *J. Agr. Sci.*, **24**, 435-456, 1934; The vitamin A requirement of swine, *ibid.*, **25**, 217-230, 1935.

cited are necessary to provide any marked storage. Considering yellow corn to contain 7 units of vitamin A per gram, a ration containing 25 per cent of this feed will meet the minimum requirements for growth. Five to eight per cent of bright green, leafy alfalfa meal will also meet the requirement.

#### OTHER VITAMINS

While there are several other vitamins which have been demonstrated to have growth functions, there is little to be said about them not covered by the general statements in Chap. VIII, either because they are known not to be needed by farm animals or because their possible requirements have not been studied.

There is ample evidence that vitamin C is not needed by any farm animals. With the exception of the chick, very few critical studies have been made of the possible needs of farm animals for the factors which make up the vitamin B complex. Deficiency symptoms which would stimulate study have not occurred in practice, and marked symptoms are hardly to be expected, even though there be physiological need, because of the rather wide distribution of these factors in the commonly used feeds.

**276. Vitamin B (B<sub>1</sub>).—**The needs of chicks and children for vitamin B are well understood. Knott<sup>1</sup> has studied the requirements of children by assaying the food and excreta with rats and thus noting the retentions with different levels of feeding. Forty Sherman units per kilogram per day was concluded to be the optimum.

Foot, Golding, and Kon<sup>2</sup> have briefly reported an experiment with pigs in which one group received a basal ration consisting of polished rice, casein, paper pulp, minerals, and cod-liver oil and in which another group received the same ration plus yeast. No growth was obtained in rats on the basal ration, and it resulted in failure in the pig also accompanied by paralysis. In contrast, the ration supplemented with yeast caused satisfactory growth in both species. These results suggest that the pig needs some factor of the B complex. This suggestion is not borne out, however, by some briefly reported results by Martin.<sup>3</sup> The

<sup>1</sup> KNOTT, ELIZABETH M., A quantitative study of the utilization and retention of vitamin B by young children, *J. Nutrition*, **12**, 597-611, 1936.

<sup>2</sup> FOOT, A. S., J. GOLDING, and S. K. KON, A note on requirements of the pig for vitamin B complex, *J. Soc. Chem. Ind.*, **50**, 442-443, 1931.

<sup>3</sup> *Forty-third Ann. Rept. Ark. Agr. Expt. Sta. Bull.* 268, p. 38, 1931.

studies indicating that the cow can synthesize some B factor in its rumen and thus may not need a dietary supply even if there is a physiological requirement have been mentioned (Sec. 161).

The needs of poultry for the antineuritic factor are readily met from commonly used feeds. Since the whole grains and their by-products which are fed to animals are generally rich sources of this vitamin, and since good quality forage, green or dry, is a substantial source also, the likelihood of any deficiency occurring in their rations seems rather remote. Nevertheless until definitely proved to the contrary, the possibility exists that farm mammals require vitamin B even as do rats and man and that their nutrition is frequently suboptimum because of a deficiency in the ration, even though there are no recognizable symptoms. From this point of view critical studies are worth while.

**277. Flavin Factor.**—The requirements of poultry for the growth factor, flavin, which most investigators have called vitamin G, are well understood. Norris and associates<sup>1</sup> state the requirement for growing chicks as 290 micrograms of flavin per 100 g. of feed. The requirement is closely correlated with growth and thus with feed intake. The requirement can be supplied by 15 per cent of dried skim milk or 10 per cent of dried whey in a ration otherwise devoid of the vitamin. The distribution of this factor in the commonly used feeds has been discussed (Sec. 168). The fact that milk is one of the richest sources of this vitamin suggests that the factor is probably required by mammals generally, but specific information is available only in the case of the rat. There is strong presumptive evidence that it is required by children. No studies of its need by farm animals other than poultry have been made. Such studies are certainly needed even though the probability exists that the commonly fed rations are not deficient because of the widespread occurrence of the vitamin.

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## CHAPTER XIV

### REPRODUCTION

While it has long been appreciated that regular and normal reproduction is the essential basis of a successful animal industry, including commercial milk production, it is only within comparatively recent years that the various aspects of this primary physiological function have received detailed study. With the recognition of infectious abortion as a major cause of reproductive troubles, concentration on the disease aspects was a natural development with the result that other phases were in part neglected. It is now appreciated, however, that the reproductive function is conditioned by a long series of distinct but interrelated physiological events in which the body as a whole as well as the sex organs is concerned and that important, though less obvious causes of failure, reside in abnormalities which are not the result of infectious disease. The economic importance of these less obvious causes has been stressed by Marshall and Hammond<sup>1</sup> as follows:

Low fertility and sterility of a temporary nature, because of their prevalence, are the cause of much greater loss to the breeding industry than infertility of a more permanent kind which occurs less frequently, although the latter, because of its striking effects, generally attracts most attention.

It is self-evident that nutrition must play at least a general role in the development and functioning of the organs of reproduction, but its significance is much larger than this. Recent discoveries have shown that specific nutrients have specific functions in the various events which comprise the reproductive cycle. To understand these functions some knowledge of the physiology of reproduction is required. This discussion which immediately

<sup>1</sup> MARSHALL, F. H. A., and JOHN HAMMOND, The physiology of animal breeding, with special reference to the problem of fertility, *Ministry Agr. Fish. Eng. Research Mon.*, 2, 1-45, 1926.

follows deals with mammalian reproduction and its nutritive requirements. At the close of this chapter the avian process is considered from the standpoint of the nutritive needs for egg production.

### PHYSIOLOGY OF REPRODUCTION

The sexual organs reach their full development and become functional at an age which varies with the species, the breed, and the nutrition of the individual. The development of these organs is a rather gradual process controlled by a secretion from the pituitary, the gland which also secretes a substance controlling body growth (Sec. 245). Apparently the gland cannot provide both secretions at a maximum at the same time, and thus the development of the sex organs proceeds more rapidly as the rate of growth declines. These organs become functional before body growth is completed. If their ability to function is immediately utilized, there may be an unfavorable effect on body development because of an undue diversion of the secretions of the pituitary from body growth to sexual activity. The lactation which follows premature breeding is one of the chief causes of the arrest of growth in the female. If the male is subjected to heavy service too early his growth and vigor are likewise impaired. Herein lies the explanation of the deleterious effects on growth and development which are recognized to occur in practice from premature breeding.

**278. Ovulation and Fertilization.**—Sexual maturity in the male is characterized by the full development of the testicles and the production of viable sperm which become motile when mixed with the secretions of certain accessory organs. In the female the functional development of the ovary is followed by a recurring cycle of events, an early stage of which is characterized by the onset of heat, or oestrus. The ovary contains a large number of minute eggs, each enclosed in a *Graafian* follicle. As the animal comes in heat one or more of these follicles enlarge, the egg is matured and liberated and then passes down the *Fallopian* tubes or oviducts to the uterus. It is during this passage that fertilization usually occurs provided the animal has been served during the heat period. With the shedding of the egg, the cavity of the *Graafian* follicle becomes filled with the *corpus luteum* or yellow body. While this yellow body is present no more follicles are

ripened and thus no more eggs are matured. If fertilization has occurred, the *corpus luteum* normally persists during the ensuing pregnancy, and thus no new fertilization can take place until its termination. If pregnancy does not occur the corpus luteum usually degenerates after a brief period, allowing a new follicle to mature as the start of a new cycle. Sometimes the yellow body persists without pregnancy, which is one of the causes of delayed breeding.

The ovarian changes of the oestrus period are accompanied by cyclic changes in the epithelial lining of the vagina. A study of these changes in the rat and guinea pig by the *vaginal smear* technique<sup>1</sup> has proved very useful as an indicator of the regularity of oestrus and of the ovarian processes involved, as influenced by various nutritional and other factors. Less well-defined cyclic, vaginal changes have been noted also in the cow, sow, and bitch. Little is known regarding them in the ewe and mare.

**279. Fetal Growth.**—The fertilized egg is nourished for a short time by secretions from glands of the uterus, and, during this time, it develops the placenta by which it becomes attached to the uterine walls. Following this implantation, it receives its nourishment from the maternal blood through its placenta and umbilical cord. The blood vessels are among the first permanent structures in the embryo, providing for the circulation of nutrients and the removal of waste products through interchange with the maternal blood. This interchange, including the oxygenation of the fetal blood, occurs in capillaries in the placenta. While the fetus receives most of its nutrients preformed, it certainly carries on some synthetic functions in connection with its growth.

Expressed arithmetically, the growth of the fetus takes place at an increasing rate throughout the gestation period. More than half of the period elapses before the weight of the fetus equals that of its membranes, whereas, at term, the placenta make up only about 20 per cent of the total weight of the products of conception. Most of the growth takes place in the last third of the gestation period, as is illustrated by the curves in Fig. 29 taken from the studies of Mitchell and associates<sup>2</sup> with swine.

<sup>1</sup> STOCKARD, CHARLES R., and GEORGE N. PAPANICOLAOU, The existence of a typical oestrous cycle in the guinea-pig—with a study of its histological and physiological changes, *Am. J. Anat.*, **22**, 225–265, 1917.

<sup>2</sup> MITCHELL, H. H., W. E. CARROLL, T. S. HAMILTON, and G. E. HUNT,

These workers slaughtered pregnant gilts in groups of one to three at weekly intervals from the fifth to the sixteenth week of gestation and determined the nutrients stored in the fetus and the placenta. The data were corrected to a standard litter of eight and treated mathematically to provide curves showing the increase in nutrient storage over the gestation period.

It is evident from Fig. 29 that approximately half of the protein and more than half of the energy storage occurs in the last quarter of pregnancy. Additional data showed that even larger

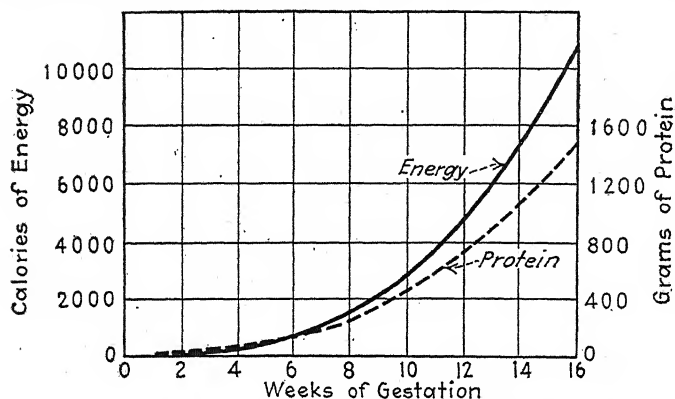


FIG. 29.—Gross energy and protein in products of conception at different stages of gestation—sow. (Mitchell, Carroll, Hamilton, and Hunt.)

proportions of the calcium and phosphorus are stored toward the end of the period. In accordance with the general picture in growing organisms, the percentage of water in the fetus decreases with age. On a dry-matter basis, protein makes up about two-thirds of the products of conception, a figure which shows little change over the gestation period. Neither do the percentages of fat or iron change markedly, but the calcium and phosphorus contents make up an increasing percentage with age. It is, therefore, clear that the quantitative demands for nutritive material are small in early pregnancy and that they progressively increase to become several times as large toward the close of the period.

In multiparous animals, the larger the number of fetuses the smaller the individuals tend to be, owing to crowding, and there

is frequently a marked difference in size among the individuals of a litter. In animals which may give birth to one or more young, multiple births do not produce so large individuals as do single births.

For each species there is a certain duration of pregnancy which is recognized as normal. Its termination is associated with the degeneration of the *corpus luteum* and is probably under the influence of both nervous and endocrine factors. The delivery of young is followed by the expulsion of the placenta or afterbirth.

#### NUTRITIVE REQUIREMENTS

Nutritional factors play vital roles in the various physiological events which occur in the attainment of sexual maturity and in the course of the reproductive process. Undernutrition delays puberty in both the male and female, and it may cause retrogressive changes in the sex organs after they are fully developed. It is a well-recognized fact that half-starved animals are relatively infertile. As a result of the inadequate food supply during and following the World War there was a large increase in sterility in cattle, sheep, and goats in Germany and Austria. The practice of flushing ewes, which is much used in England and Scotland, arose from the observation that the lamb crop was increased when ewes kept under range or semirange conditions were heavily fed a week or two before the breeding season. Apparently irrespective of the nature of the feed, flushing stimulates ovarian activity and oestrus, and increases the number of eggs ripened by preventing the degeneration of maturing follicles which otherwise occurs as a result of undernutrition. If the severely undernourished animal becomes pregnant, the drain on her body by the developing young may result in permanent damage. The death of the fetus *in utero* or the birth of a weak animal, perhaps prematurely, may also occur. In the male, undernutrition decreases the number and vigor of the sperm.

A condition of extreme fatness is also deleterious to reproduction. The ovaries may become so infiltrated with fat as to hinder the development of the follicles, with a consequent irregularity or cessation of oestrus which results in delay or failure in breeding. There may be also such an excessive amount of fat in the reproductive tract that, even if the egg is matured and fertilized, it may fail to reach the uterus and become implanted properly.

Extreme fatness also interferes with the production of fertile sperms in the male and lessens his desire to mate. It is recognized that show condition is associated with low fertility in both sexes.

While the nutritive intake is qualitatively of equal importance in both sexes, obviously the quantitative requirements are vastly greater for the female. Thus most of the following discussion deals with the needs of the mother. Her nutrition must have the double object of producing normal offspring and of protecting her own tissues, for on an inadequate ration the mother sacrifices her bones and other tissues to nourish her fetus.

**280. Energy Requirements.**—While the effects of general undernutrition previously mentioned are undoubtedly the result of more than one deficiency, it is evident that the energy supply is an important factor. Experiments with rats have shown that a deficiency of energy alone results in a delay in the opening of the vagina, a prolongation of the period between this event and the first oestrus, and an irregularity or cessation of the oestrus cycle. The studies by Asdell and Crowell<sup>1</sup> showing these effects included observations on sexually mature rats which were held at constant weight far below normal size, by energy restriction. Under these conditions, the cycles were highly irregular. When the animals were given an increment of energy sufficient to cause some growth, regular cycles occurred until the animals reached a weight where growth ceased because the total energy intake was needed for maintenance. Here sexual activity ceased also. Thus it was shown that neither growth nor sexual activity takes precedence over the other.

The energy requirement for reproduction consists of the energy stored in the new tissue formed plus the energy expended in the process. The tissues formed include the fetus and its membranes, the enlargement of the uterus, and the mammary development (Sec. 298). The energy content of these tissues at different stages provides the basic figures for estimating the nutritive requirements over the gestation period. Mitchell and associates<sup>2</sup> in their studies, previously cited, with swine, computed the daily increase in energy and other nutrients in the products of concep-

<sup>1</sup> ASDELL, S. A., and MARY F. CROWELL, The effect of retarded growth upon the sexual development of rats, *J. Nutrition*, 10, 13-24, 1935.

<sup>2</sup> MITCHELL, CARROLL, HAMILTON, and HUNT, *loc. cit.*

tion. These data are reproduced in Table XXVIII. It is noted that the energy storage is very small during the early weeks. While 272 Cal. are stored daily during the last week, computation shows that the average daily deposition for the period as a whole is only 104 Cal. No data are available for estimating the energy stored in the mammary growth, but, except in a first pregnancy,

TABLE XXVIII.—COMPUTED DAILY RATE OF INCREASE IN WEIGHT AND ENERGY CONTENT, AND COMPUTED DAILY DEPOSITION OF NUTRIENTS IN THE UTERI OF PREGNANT GILTS

Week of gestation	Total weight, g.	Gross energy, Cal.	Crude protein, g.	Ash, g.	Calcium, g.	Phosphorus, g.	Iron, mg.
1	27	1.6	0.54	0.028	0.0001	0.0011	0.28
2	49	5.9	1.5	0.126	0.0018	0.0074	0.71
3	71	12.5	2.7	0.30	0.0081	0.022	1.24
4	91	21	4.2	0.57	0.024	0.048	1.84
5	111	32	5.9	0.93	0.055	0.087	2.50
6	131	45	7.7	1.38	0.109	0.142	3.2
7	150	59	9.6	1.93	0.194	0.215	4.0
8	169	76	11.8	2.6	0.32	0.31	4.8
9	187	94	14	3.3	0.50	0.42	5.6
10	205	115	16	4.3	0.74	0.56	6.5
11	224	137	19	5.2	1.05	0.72	7.4
12	242	160	21	6.2	1.46	0.91	8.3
13	259	186	24	7.4	1.97	1.13	9.2
14	277	213	27	8.7	2.60	1.38	10.2
15	294	242	30	10.1	3.37	1.67	11.2
16	312	272	33	11.7	4.29	1.98	12.3

it should not exceed 10 per cent of that in the uterine products. On this basis a daily intake of 115 Cal. could be considered as the net-energy requirement for reproduction in a sow producing a litter of eight pigs. That these calculations are only approximations is clear, but the final value obtained is useful for comparison with the estimated net-energy requirement for maintenance.

The gilts studied by Mitchell weighed around 200 lb. Armsby gives the net-energy requirement for maintenance of a pig of this weight as 1.99 Therms. Even on the basis of this figure, which is probably too low, the average daily need for reproduction itself



adds only 6 per cent to the maintenance requirement and less than 15 per cent during the last week of gestation when the demand is greatest. Approximately the same relations should hold for the requirements in terms of digestible nutrients. Despite the possible errors involved in these computations, they show that even at the period of maximum demand reproduction does not involve a large increase in energy intake over the maintenance requirement and that, for the early part of the gestation, the additional need is negligible.

Because of the relatively small size of the energy need for reproduction and its variability, no attempt is made in practice to consider it separately from the maintenance requirement. In feeding standards, figures are given for pregnant animals to cover the needs for both maintenance and reproduction. It is recognized that in practice the condition of the animal must be considered. The energy supply should be sufficient to keep the animal in good flesh without becoming too fat. Clearly there should be a marked gain in weight during the last part of the gestation. This should involve some storage in the pregnant animal's own tissues as well as in the fetus since body reserves are frequently called upon during the height of the lactation which follows parturition (Sec. 315). When the animal is in a thin condition at the start of the gestation as a result of previous lactation or of underfeeding, the energy supply must obviously be larger in order to restore lost flesh and to provide the desired reserves. The immature animal which is bred must have a supply of food which will enable it to continue its growth as well as to nourish its fetus. Under this condition, the energy requirement for growth is much larger than for the reproductive function.

**281. Fat Metabolism in Reproduction.**—A specific requirement for fat for reproduction in the rat has been reported by Burr and Burr (Sec. 73) who found that, on a fat-free diet, ovulation became irregular or ceased entirely, a disturbance which could be corrected by adding an appropriate fat. The males deprived of food fat showed a lack of sex interest and, with few exceptions, would not mate. It is probable that, if any such need for fat exists for farm animals, it is always taken care of in practical rations, since these rations of natural foods are never free from this nutrient nor from the specific fatty acids which Burr and Burr have found essential.

Many experiments, several of which have been reviewed by Tyler and Underhill<sup>1</sup> in their report of a study with women, show that various lipids play specific and augmented roles in reproduction. The phosphorus content of the *corpus luteum* of the sow increases two or three times during the period of activity prior to oestrus and during pregnancy. The blood lipids increase in various species during gestation. They are higher in the hen than in the cock and highest in the female during the laying period, with the peak at the time of ovulation. These findings do not necessarily imply a specific dietary requirement for any lipid, but they do suggest the importance of further studies of lipid metabolism in reproduction.

**282. Protein Requirement.**—When the protein intake is deficient, the oestrus cycle is upset. Since the dry matter of the products of conception consists largely of protein, it is evident that there is a substantial requirement for this nutrient, and it is also clear from experiments with laboratory animals that the quality of the protein ingested is of equal importance here as in growth. The data in Table XXVIII show that the quantitative need does not become of large importance until the last half of pregnancy during which the daily storage increases rapidly. The average daily figure for the gestation period as computed from these data is approximately 14 g. Assuming that this should be increased to 16 g. to include the mammary development and that the digestible protein fed has a biological value of 50 per cent, the average daily requirement for the sow producing a litter of eight would be 32 g. Similarly computed, the figure for the last week of gestation would be approximately 70 g. According to Brody's estimate<sup>2</sup> from endogenous nitrogen a 200-lb. animal should receive approximately 100 g. of protein daily for maintenance. On this basis the pregnant sow requires an average increment of 32 per cent throughout gestation, the requirement being over twice this at the close of the period. Similar calculations for the cow, based on meager data, indicate that the average daily gesta-

<sup>1</sup> TYLER, MARGARET, and FRANK UNDERHILL, The influence of pregnancy on the lipids of the blood, *J. Biol. Chem.*, **66**, 1-13, 1925.

<sup>2</sup> BRODY, SAMUEL, ROBERT C. PROCTER, and URAL S. ASHWORTH, Growth and Development. XXXIV. Basal metabolism, endogenous nitrogen, creatinine and neutral sulphur excretions as functions of body weight, *Mo. Agr. Expt. Sta. Research Bull.* 220, 1934.

tion demand increases the maintenance requirement by 17 per cent, with a figure of 40 per cent at the end of the period. These calculations show that pregnancy increases the need for protein much more than for energy. This means that the ration fed during gestation should have a narrower nutritive ratio than is required for maintenance alone.

Studies with different species have shown that the pregnant animal has a considerable capacity to store protein in its own body as well as in the products of conception. This has been demonstrated for women by Hunscher and associates.<sup>1</sup> They measured the total nitrogen retained by intermittent balance studies over the period of gestation, and the nitrogen in the fetus and adnexa was calculated from reported analyses. The data thus obtained revealed a marked retention in excess of that required for fetal development. Their data showed that following parturition the mother was in negative nitrogen balance during the first two weeks of lactation despite a liberal protein intake. Similar findings have been reported for cows and other species. These various observations emphasize the importance of a protein intake during gestation, in excess of the minimum requirements for maintenance and fetal growth, to take full advantage of the capacity of the maternal organism to store reserves for the period immediately following parturition when the food supply may be incapable of meeting the demands for lactation.

**283. Effects of Calcium and Phosphorus Deficiencies.**—The observations in the phosphorus-deficient areas (Sec. 110) throughout the world are in agreement that reproductive troubles are very common and that they have caused very large losses in the animal industry. Conclusive proof for cows is furnished by the extensive studies of Theiler and associates<sup>2</sup> carried out in the phosphorus-deficient area in South Africa. The studies included observations on 200 animals over a period of two years. In groups in which the phosphorus-deficient pasture was supple-

<sup>1</sup>HUNSCHER, HELEN A., EVA DONELSON, BETTY NIMS, FANNY KENYON, and ICE G. MACY, Metabolism of women during the reproductive cycle. V. Nitrogen utilization, *J. Biol. Chem.*, **99**, 507-520, 1933.

<sup>2</sup>THEILER, A., H. H. GREEN, and P. J. DU TOIT, Studies in mineral metabolism. III. Breeding of cattle on phosphorus deficient pasture, *J. Agr. Sci.*, **18**, 369-371, 1928; Phosphorus in the live stock industry, *J. Dept. Agr., Union S. Africa*, **8**, 460-504, 1924.

mented by bone meal or other phosphorus sources, the calf crop was approximately 80 per cent in contrast to a figure of approximately 51 per cent in the control group. Similar evidence has been presented from various areas in the United States. The most frequently observed specific trouble is irregularity or cessation of oestrus, corresponding to the commonly reported finding in rats.

It is becoming apparent, however, that other factors, perhaps a lack of vitamin A, are also concerned in some of the reproduction troubles in phosphorus-deficient areas. Eckles and coworkers,<sup>1</sup> in a study undertaken as a result of their observations of reproductive troubles in cattle in the phosphorus-deficient areas of Minnesota, came to the conclusion that the troubles could not be attributed to a lack of phosphorus alone. They found that uncomplicated phosphorus deficiency, produced experimentally in mature dairy cows and continued for two or three years, did not disturb oestrus, although it did appear to reduce breeding efficiency.

Calcium deficiency can also upset reproduction. It has been found to cause intra-uterine death in rats and pigs. The failure appears to be due to a lack of tone of the uterine muscle. A lack of calcium is much less an area problem than is the case for phosphorus.

Though of primary importance, regularity of breeding and a normal pregnancy and parturition do not constitute complete proof that the calcium and phosphorus nutrition is adequate for reproduction. Despite a normal birth there may be pathological changes in the osseous system of the newborn as a result of mineral deficiency in the diet of the mother. Of equal importance, the mother's bones may be depleted to supply the minerals in the skeleton of the fetus. This has been shown to occur in various species of animals fed rations low in calcium and phosphorus. To the extent that it involves only the reserves of the minerals in the bones, no structural injury is caused, but, since in the lactation to follow, the demand for these minerals is so large that losses from the bones cannot be prevented despite the

<sup>1</sup> ECKLES, C. H., L. S. PALMER, T. W. GULLICKSON, C. P. FITCH, W. L. BOYD, L. BISHOP, and J. W. NELSON, Effects of uncomplicated phosphorus deficiency on estrous cycle, reproduction, and composition of tissues of mature dairy cows, *Cornell Vet.*, 25, 22-43, 1935.

most liberal nutrition (Sec. 325), it is clearly desirable to husband the reserve in the bones during gestation. It has been shown with sheep by Fraser and associates<sup>1</sup> and with swine by Evans<sup>2</sup> that losses from the bones which occur on a deficient ration can be prevented, by increasing the intakes of the bone-forming minerals. Similar findings have been reported from studies with women and with rats. Severe or continued depletion of the bones results in osteomalacia (Sec. 110).

The fact that the mother's bones can be sacrificed in the interests of the fetus provides a means of protecting the offspring of a first pregnancy from serious skeletal defects. Coons and Blunt,<sup>3</sup> however, obtained evidence by X-ray studies that calcification was best in infants from mothers showing the highest retentions of calcium and phosphorus during pregnancy. In a study with sows fed a ration deficient in calcium, Davidson<sup>4</sup> found that a calcium-deficient ration did not produce an immediate effect because of the store in the maternal body. In successive farrowings there was an increase in the number of pigs born weak or dead and a decrease in the number reared to weaning. There was a serious reduction and eventual failure of the milk supply. Several investigators have reported fetal rickets, gross hypoplasia of the enamel, and defective dentine in infants from mothers undernourished in bone-forming minerals during pregnancy.

**284. Calcium and Phosphorus Requirements.**—In addition to providing for the growing fetus, the calcium and phosphorus intakes during gestation must be sufficient to meet the maintenance requirement of the mother and also to build up reserves in her bones in so far as this is possible. Data as to the amounts of these minerals present in the products of conception at birth are of limited value in arriving at the actual intake needs for several

<sup>1</sup> FRASER, A. H. H., W. GODDEN, and W. THOMSON, The effect of a calcium-deficient diet on pregnant ewes, *Vet. J.*, **89**, 408-411, 1933.

<sup>2</sup> EVANS, R. E., Protein and mineral metabolism in pregnant sows on a normal or high calcium diet, compared with a calcium-deficient diet, *J. Agr. Sci.*, **19**, 752-798, 1929.

<sup>3</sup> COONS, CALLIE MAE, and KATHARINE BLUNT, The retention of nitrogen, calcium, phosphorus, and magnesium by pregnant women, *J. Biol. Chem.*, **86**, 1-16, 1930.

<sup>4</sup> DAVIDSON, H. R., Reproductive disturbances caused by feeding protein-deficient and calcium-deficient rations to breeding pigs, *J. Agr. Sci.*, **20**, 233-264, 1930.

reasons. For most species there is a lack of information as to the maintenance requirement, and there is no reliable figure as to the percentage retention which can be assumed where it is desired to insure storage in the mother's bones to the fullest extent that it is possible, as well as in the fetus. The efficiency of utilization is recognized to be low under these conditions. Further, the daily requirement for fetal growth cannot be based upon the average storage in products of conception, for almost all of this storage takes place in the last half of gestation and especially in the last fifth. This fact is clearly brought out in Table XXVIII. The intake chosen for the period as a whole must be that which will be optimum for the last days of gestation, or provision must be made for increasing a lower initial intake in accordance with increasing storage.

While extensive data are available for rats, few studies have been made with farm animals providing information as to the minimum intakes of calcium and phosphorus which can be relied upon to be optimum over the gestation period alone. In a very extensive balance experiment with swine, Evans<sup>1</sup> compared intakes of approximately 0.6 and 0.04 per cent of calcium in a basal ration adequate in other nutrients including vitamin D. On the low level of intake only 15 g. of calcium was retained during gestation although five times this amount was found in the products of conception at the close. A large depletion of the sow's skeleton therefore occurred. There was a lack of milk secretion following farrowing. The high level resulted in a storage in gestation greatly in excess of the demand for fetal growth, demonstrating the ability of the maternal organism to build up her reserves when the dietary supply is sufficiently large. From records of the number of pigs farrowed and weaned and from studies of the bones of the mothers, Hogan<sup>2</sup> has suggested that the ration of brood sows should not contain less than 0.4 per cent of calcium. The striking effect of an inadequate supply on the bones of the gilt, noted by Hogan is shown in Fig. 30. Probably 0.3 per cent of calcium will provide an adequate supply for the mature pregnant sow up to the middle of gestation, but then it must be increased, and the ration should contain at least 0.5

<sup>1</sup> EVANS, *loc. cit.*

<sup>2</sup> HOGAN, A. G., The calcium requirement of brood sows, *Mo. Agr. Expt. Sta. Research Bull.* 167, 1932.

per cent at the close. Gilts need larger amounts in view of their growth requirements. Somewhat less phosphorus than calcium is needed.

In terms of body size, pregnant cows have a lower requirement for bone-forming minerals than do swine. Evidence that very low levels of calcium may not interfere with reproduction is furnished by studies of Palmer and associates.<sup>1</sup> Cows which reproduced normally for three years on a ration containing only 0.18 per cent of calcium continued to do so for two years more when the content was reduced to 0.12 per cent. The ration did cause some depletion of the bones. Since in dairy cows, pregnancy is superimposed upon lactation, except in the case of the first gestation, it is only for the latter that separate figures are

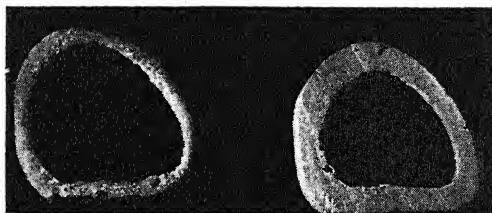


FIG. 30.—Cross section of metacarpal bones. The bone on the left was taken from a gilt on a low-calcium ration. Note the thin-walled, spongy, porous condition compared to the bone on the right produced on an adequate ration. The poor bone contained only half as much calcium. (Courtesy of A. G. Hogan, University of Missouri.)

particularly useful. Here growth is also concerned. The requirements of dairy heifers up to first calving have been previously discussed (Sec. 269). The needs of animals which are both lactating and pregnant are considered later (Sec. 327).

For ewes, Riches and Godden<sup>2</sup> found that the average daily storage in the lamb and its membranes was 0.6 g. of calcium. On an intake which supplied double this amount daily the calcium balance was negative. An intake of 6 g. daily was certainly adequate. Probably it was unnecessarily high. A ration for ewes containing 0.2 per cent each of calcium and phosphorus

<sup>1</sup> PALMER, L. S., C. P. FITCH, T. W. GULLICKSON, and W. L. BOYD, Supplementary report of an experiment to determine the effect of a low calcium ration on reproduction in cattle. Effects of further reduction in calcium and of removing vitamin supplements, *Cornell Vet.*, 25, 229-246, 1935.

<sup>2</sup> Cited in GARRY, R. C., and D. STIVEN, A review of recent work on dietary requirements in pregnancy and lactation, with an attempt to assess human requirements, *Nutrition Abs. Rev.*, 5, 855-887, 1935-6.

should suffice until the last third of gestation when it should be increased to provide levels up to 0.35 per cent at the close. It seems probable that the needs of the mare are similar to those of the ewe. In the case of all species, the demands are greater where a pregnancy occurs before growth is completed.

**285. Vitamin D.**—The data are rather fragmentary or conflicting as to the extensiveness of the role of vitamin D during reproduction. In the case of rats, most studies have reported an increased calcium and phosphorus retention during pregnancy and an increased content of the minerals and of the vitamin in the newborn, where liberal intakes of the factor have been provided. Several studies have been carried out with women. In some cases the calcium and phosphorus balances have been improved by additions of the vitamin, in others not. It is generally agreed that the newborn infant from a mother who has received a liberal intake of the antirachitic factor is less susceptible to rickets, because of the storage of the vitamin in the fetus. This storage is not sufficient to have any prolonged antirachitic effect in the offspring. There is practically no exact information regarding the influence of vitamin D during gestation in farm animals. As is discussed later (Sec. 332), no form of the factor has been found effective in lessening the negative mineral balances which may occur in dairy cattle during lactation, or in increasing the storage which normally takes place during the dry period which also generally coincides with the time of greatest demand for gestation.

**286. Iron.**—The studies by Mitchell showed that the sow producing a litter of eight stored 580 mg. of iron in the products of conception. During the last week of pregnancy, the daily storage was 12.3 mg. (Table XXVIII), and the average figure for the period as a whole was 5.5 mg. Even the latter figure is greatly in excess of the maintenance requirement. Studies with women have also indicated that the demands for fetal growth are much greater than those for maintenance. It is probable that, for all species, the maintenance requirement must be increased two or three times to cover the needs of the gestation.

If the intake during pregnancy is deficient, the needs of the fetus are supplied from the mother's reserves in her liver and spleen, but, if the deficiency is extreme, the amount stored in the newborn will be less. This has been shown in rats by Lintzel and



Radeff.<sup>1</sup> Pregnant rats, receiving a normal ration containing 11 mg. of iron per 100 g., produced litters in which the average iron content of the individuals was approximately 0.25 mg. This storage figure was reduced by one-quarter to one-half when the mother's diet was nearly iron-free. The mother's reserves were largely depleted and hemoglobin iron was lowered. While the normal picture in the mother and young was restored by iron addition, the amount of iron stored in the young by the ration containing 11 mg. was not increased by doubling the dietary level.

Despite the very large increase in the iron requirement which accompanies pregnancy, there is no evidence that a practical problem is here presented in the case of farm animals. In terms of the need, the commonly used feeds are rich (Sec. 123). It seems very unlikely that any deficiency exists, but the question may deserve critical study in swine in view of the common occurrence of anemia in the suckling young. Doubtless this trouble can occur even though the physiological capacity to store iron in the fetus is reached, but there may be dietary situations where the maximum storage does not take place.

**287. Iodine.**—The occurrence of goiter in farm animals as a result of a deficiency in the diet of the mother during gestation has been discussed (Sec. 124). There are no exact data as to minimum amounts of iodine required to insure a normal birth, but experience in those areas where goiter is endemic has established intakes which can generally be relied upon to prevent its development. The additional iodine needed is commonly provided as iodized salt, potassium, or sodium iodide, or some other compound. The feeding of the iodine supplement during the last three-quarters of the gestation period is sufficient. The use of iodized salt (0.02 per cent potassium iodide) has been found effective for all classes of farm animals in goiter areas. One must be certain that the iodine is actually present. Iodized salt loses some of its iodine if stored for a considerable time at warm temperatures, or if exposed to sun and rain. Rodenwold and Simms<sup>2</sup> have reported that 5 grains of potassium iodine per week is an

<sup>1</sup> LINTZEL, W., and T. RADEFF, Über den Eisengehalt und Eisenansatz neugeborener und saugender Tiere (nach Versuchen an Kaninchen, Meer-schweinchen, Ratte, Hund, Katze, Schwein, Ziege, Rind), *Archiv. Tierernähr. Tierzucht.*, 6, 313-358, 1931.

<sup>2</sup> RODENWOLD, B. W., and B. T. SIMMS, Iodine for brood mares, *Proc. Am. Soc. of Animal Production*, 1934, pp. 89-92.

adequate intake for the cow. Limited data indicated that an intake of 15 grains produced stronger and more vigorous foals than where 5 grains were fed. Some puzzling instances of failure to respond to treatment have been noted for the mare. One or two grains weekly have been recommended for sows and from 0.35 to 1 grain for ewes. It is probable that current recommendations are higher than necessary rather than otherwise. Studies as to exact requirements are needed.

There is no conclusive evidence that the feeding of additional iodine to breeding animals is helpful except in the specific situations where goiter is occurring, or that it has any benefits other than goiter prevention. There are some reports that the element has a favorable effect on oestrus and that it will even lessen abortion troubles. These reports require further confirmation, particularly as there have been well-conducted experiments in which no such beneficial effects have been found (Sec. 126). Since the danger of overdosage with iodine is a real one, it seems wise to restrict its use to the prevention of goiter and related troubles in areas where they otherwise occur, until positive benefits for other purposes have been clearly proved.

**288. Vitamin A.**—In all species, in so far as studied, the reproductive process is dependent upon an adequate supply of vitamin A. This is true for both sexes. In the male, a deficiency of the vitamin causes a very rapid degeneration of the germinal epithelium of the testes with a resultant loss of fertility. The degeneration occurs more rapidly than in vitamin E deficiency (Sec. 289). In the female there may be, in the first place, a cessation or irregularity of oestrus. This disturbance of the cycle is accompanied by a cornification of the vaginal epithelium which can be detected in the rat by the smear technique (Sec. 278) thus providing a means of early diagnosis. The extent of the deficiency determines whether there is a complete failure to breed or whether, after a delay, oestrus occurs. If fertilization actually takes place, an injury to the placenta may occur which results in fetal death and resorption, or in an abortion. In certain instances the gestation is prolonged and terminates in a difficult parturition. These various effects have been described in detail in the rat by Mason<sup>1</sup> who differentiates the pathological changes here involved

<sup>1</sup> MASON, KARL E., Fetal death, prolonged gestation and difficult parturition in the rat as a result of vitamin A deficiency, *Am. J. Anat.*, **57**, 303-344, 1935.

from those causing fetal death in vitamin E deficiency (Sec. 289). The latter primarily affects the fetal tissue, whereas, when vitamin A is lacking, fetal death is secondary to placental injury and to alterations of the uterine epithelium.

The symptoms which occur differ somewhat according to the severity of the deficiency and some may be more prominent in one species than another. In cattle, Hart and Guilbert<sup>1</sup> have reported the birth of dead or weak calves with frequent retention of the placenta, a condition which simulates infectious abortion. Severe diarrhea resembling white scours was present in the weak, newborn calves. These results occurred in animals, negative to the blood test for abortion, which had been maintained for an extended period on dried-up range. Following a failure during gestation, the animals commonly did not come in heat again until they had access to green feed. In sows, a disturbance of the oestrus cycle and the farrowing of premature, weak, or dead pigs have been reported by Hughes and associates.<sup>2</sup> In sheep the lambs die *in utero* or soon after birth. Animals which fail in reproduction because of a deficiency of vitamin A may or may not show the eye, nerve, and other symptoms (Sec. 141) which commonly occur during growth, depending upon the severity of the deficiency. Intakes which are sufficient to prevent eye symptoms may cause disaster during gestation. This indicates that the needs for reproduction are greater than for maintenance.

Little is known as to the minimum requirements of vitamin A for successful reproduction. Guilbert and Hart<sup>3</sup> found 15 micrograms of carotene per kilogram per day insufficient in the case of the cow, whereas 30 micrograms, tripled during the last month, proved adequate in the one case tried. The swine ration which is based upon yellow corn should provide the vitamin adequately, but rations based upon white corn or barley have been proved deficient for reproduction. The needs of Herbivora can be taken care of readily by green, leafy roughage. The disastrous results which occur in dairy cows from the continuous feeding of

<sup>1</sup> HART, G. H., and H. R. GUILBERT, Vitamin-A deficiency as related to reproduction in range cattle, *Calif. Agr. Expt. Sta. Bull.* 560, 1933.

<sup>2</sup> HUGHES, J. S., C. E. AUBEL, and H. F. LEINHARDT, The importance of vitamin A and vitamin C in the ration of swine, *Kan. Agr. Expt. Sta. Tech. Bull.* 23, 1928.

<sup>3</sup> GUILBERT, H. R., and G. H. HART, Minimum vitamin A requirements with particular reference to cattle, *J. Nutrition*, 10, 409-427, 1935.

low-grade timothy hay in contrast to the performance on high-quality alfalfa have been clearly shown by the work of Meigs and Converse.<sup>1</sup> Later studies by these workers found vitamin A to be the factor concerned.

Fortunately, the ability of animals to store vitamin A protects them from reproductive failure during short periods on feed deficient in this factor. The most liberal intakes during gestation, however, do not result in any marked storage in the newborn.

**289. Vitamin E.**—In the male rat, the absence of vitamin E from the diet results in a failure of sperm formation because of a degeneration of germinal tissue, the seminiferous epithelial cells. These degenerative changes are irreversible and, therefore, incurable. An early external, though nonspecific, sign of deficiency is the occurrence of immotile sperms in the semen. Sex interest is maintained long after fertilizing power is lost, but this interest finally disappears with the degeneration of the secondary sex organs. A similar testicular degeneration occurs in the cock (Sec. 296).

In the female rat, a lack of vitamin E does not interfere with ovarian functions, or with the implantation of the fertilized egg. In fact, everything proceeds normally for nearly a quarter of the gestation period. Thereafter, the fetus degenerates and dies and absorption occurs, primarily as a result of injury to fetal tissues. Changes in the epithelial lining of the uterus are involved. These pathological changes in the female do not result in permanent damage. If the rat in which fetal death has occurred is given a diet containing an adequate amount of the vitamin, the following gestation will be normal. In fact, the addition of a vitamin supplement to a deficient diet can be delayed until the fourth or fifth day of gestation and the birth will be normal, although fetal death would have occurred otherwise.

The histology of vitamin E injury has been very carefully studied by several investigators. It is generally believed at the present time that this factor is needed for nuclear activity and function of cells in general and that a deficiency reveals itself primarily in those cells where proliferation and differentiation are

<sup>1</sup> MEIGS, EDWARD B., and H. T. CONVERSE, Some effects of different kinds of hay in the ration on the performance of dairy cows, *J. Dairy Sci.*, **16**, 317-328, 1933; The vitamin requirements for reproduction and lactation under practical conditions, *ibid.*, **19**, 438, 1936.

especially rapid, *viz.*, in the germinal epithelium of the testes and fetus. In view of the important role of hormones in reproduction, a relation of vitamin E to their activity has been proposed, but no definite proof has been obtained.

**290. Is Vitamin E Required in the Rations of Farm Animals?—**

Vogt-Moller and Bay<sup>1</sup> of Denmark reported a large percentage of successes by the injection of a vitamin E concentrate into dairy cows which were coming in heat regularly but failed to become settled with calf. This suggestive evidence of a need for vitamin E by the cow has not been satisfactorily confirmed thus far in other laboratories. In view of the predominant role of infectious disease in breeding troubles in dairy cows, as well as the various other physiological disturbances which may result in reproductive failure, very extensive and carefully controlled experiments will be necessary to furnish certain proof that vitamin E is required in the ration.

These Danish investigators also report that sows which failed to come in heat *post partum* responded to vitamin E. Such a response does not correspond with the effect of the vitamin in the rat, since the latter species shows oestrus regularly on diets deprived of it. It is also contrary to an earlier report by Aubel and coworkers<sup>2</sup> who failed to find any significant beneficial effect on reproduction in swine from the addition of the vitamin to a diet which caused failure in the rat. Wilson and coworkers<sup>3</sup> have been unable to demonstrate any need for the vitamin by goats. The Danish workers have also claimed positive results in the treatment of sterility and habitual abortion in women.

The production of final proof that any large mammal does or does not require vitamin E in its food presents a very hard problem, owing to difficulties in providing purified diets on an extensive scale, the large possible role of reserves, and the impossibility

<sup>1</sup> VOGT-MOLLER, P., and F. BAY, On treatment of sterility in cows with wheat germ oil (Vitamin E), *Vet. J.*, **87**, 165-170, 1931. BAY, F., and P. VOGT-MOLLER, Continued studies on treatment of sterility in cows and breeding sows with wheat germ oil (Vitamin E), *ibid.*, **90**, 288-290, 1934.

<sup>2</sup> AUBEL, C. E., J. S. HUGHES, and H. F. LEINHARDT, The influence of vitamins B and E on reproduction in swine, *Proc. Am. Soc. Animal Production*, 1929, pp. 133-135.

<sup>3</sup> WILSON, JOHN L., B. H. THOMAS, and C. Y. CANNON, The dietary requirements of goats for vitamin E (A progress report), *J. Dairy Sci.*, **18**, 431-432, 1935.

of studying specific physiological steps in reproduction as effectively as can be done for the rat. The vitamin is widely distributed in the commonly used feeds (Sec. 160) and a short period of deprivation is unimportant because of the capacity of the body to store the factor (Sec. 158). These facts suggest that, even if required in the rations of farm animals, the likelihood of there being a sufficient deficiency of the vitamin to cause reproductive failure is small. But it is unwise for the nutritionist to dismiss the matter on these grounds. The richness of by-product feeds in vitamin E depends primarily on the presence of the germ. More and more of this germ is going into other channels at the present time. Much of the cornmeal now sold for feed comes from degerminated grain. These changes are of no concern where the ration consists of farm-grown feeds, but, where commercial feeds are largely relied upon, the situation is different. Despite its difficulty, the question as to whether reproduction in our large animals is dependent upon a supply of vitamin E in their rations must be definitely answered.

**291. Other Vitamins.**—Reproductive failure in rats has been reported on diets deficient in vitamin B. It is uncertain whether this is a specific effect or the result of inanition caused by the effect of the vitamin on appetite. The possible roles of other vitamins in mammalian reproduction remain unknown.

**292. Nutrition and Diseases of Reproduction.**—By far the most important reproductive losses in farm animals, particularly in dairy cattle, are those which are due to infectious disease, notably abortion. While it is well recognized that undernutrition may make animals more susceptible to certain diseases, this phase of nutrition has been overemphasized in connection with reproduction. This idea was specifically tested for infectious abortion by Hart and associates<sup>1</sup> in a very comprehensive experiment. Cows receiving high levels of intake of calcium, phosphorus, vitamin D, and iodine proved no more resistant to the infection than animals whose nutrition was border line in these respects.

#### EGG PRODUCTION

Differing from mammals which nourish the embryo inside their bodies, give birth to living young, and nurse them, birds produce

<sup>1</sup> HART, E. B., F. B. HADLEY, and G. C. HUMPHREY, The relation of nutrition to contagious cattle abortion, *Wisc. Agr. Expt. Sta. Research Bull.* 112, 1932.

eggs which contain sufficient nutrients for the embryo to develop outside the body and no preformed food is required after hatching. In the hen the egg-formation phase of reproduction has been extended into a continuous process, aside from the moulting period, whereby egg production has become a tremendous industry as a source of human food, as well as serving in the propagation of the species.

The egg of the hen is made up approximately as follows: yolk, 31 per cent; albumen or white, 59 per cent; and shell, 10 per cent. The development of the egg starts in the ovary where the yolk portion is formed. Here there are many ova, each enclosed in a follicle. The yolk is deposited in concentric layers and when the process is completed the follicle bursts, and the yolk, surrounded by a membrane, passes into the oviduct. Here the albumen is put on and finally the shell, each process requiring several hours. During its passage through the oviduct, the developing egg is fertilized if sperm are present. After shell formation is completed, the egg passes out through the vent. Under proper temperature conditions, the fertilized egg develops into the chick in twenty-one days. The reproductive process in the cock is similar to that in mammals.

**293. Nutritive Requirements for Egg Production.**—The hen ranks with the dairy cow in her productive performance. In a year she may produce up to four times as much dry matter as is contained in her body. Clearly an intensive metabolism and very large nutritive requirements are involved. The egg has the following approximate composition: water, 66 per cent; protein, 13 per cent; fat, 10.5 per cent; ash, 10.5 per cent. These figures reveal the fact that, in addition to the energy requirement, there are large demands for protein and especially for mineral matter. There are also important requirements for various vitamins. Besides the large nutritive demands for the formation of the egg as such, there are additional requirements for the production of an egg that will hatch and yield a strong chick. The magnitude and complexity of the demands for the intensive egg production which characterizes the modern commercial practice have made its nutrition a specialized field, which can be discussed only briefly here.

The adequacy of the *protein* content of the ration is measured in terms of the number of eggs produced, their size, and the main-

tenance of the body weight of the bird. According to several studies, these requirements can be met satisfactorily when the ration contains 15 to 16 per cent of protein, provided it is of high biological value. Protein of the proper quality is provided in practice by including those products, such as skim milk, fish meal, meat scraps, soybean meal, and others, which in combination with cereals and their by-products furnish efficient protein mixtures (Sec. 259).

**294. Calcium, Phosphorus and Vitamin D.**—The average egg contains approximately 2 g. of calcium and 0.12 g. of phosphorus. Nearly all of the calcium is in the shell which consists very largely of calcium carbonate, while the phosphorus is concentrated in the yolk, principally combined with protein. Owing to the large amount needed for shell formation, the calcium requirement of the laying hen is several times that for any other species. The intensity of the metabolism involved is reflected in a doubling of the level in the blood serum during the laying period. A 4-lb. hen which lays an egg daily requires for its eggs alone twice as much calcium as the chick, many times the hen's size, needs for growth. A deficiency of the element results in thinner shells, a marked depletion of the bones of the hen, and a lowering of egg production. The same effect on the hen and upon her production occurs from a lack of phosphorus. Though this element is needed in much smaller amounts than is calcium, no mammal has as high a requirement for any purpose.

Some mobilization of calcium and phosphorus from the bones during heavy egg production appears to be a normal physiological process even as is the case for lactation (Sec. 325). This fact emphasizes the importance of building up the reserves in the growing chick and of continuing a high level of feeding at all times in the case of the hen, for the depleted bones must be restored during the period when production falls off or ceases.

According to Norris and associates,<sup>1</sup> the calcium requirement can be met by a ration containing 1.8 per cent of this element. Much of the calcium needed is commonly supplied by the feeding of oyster shells or some other special source of calcium. The

<sup>1</sup> NORRIS, L. C., G. F. HEUSER, A. T. RINGROSE, and H. S. WILGUS, JR., Studies of the calcium requirements of laying hens, *Poultry Sci.*, **13**, 308-309, 1934.



phosphorus requirement has been determined by Miller and Bearse<sup>1</sup> as 0.80 per cent.

No amounts of calcium and phosphorus will be effective without an adequate supply of vitamin D also. The vitamin exerts an important influence, not only on the number of eggs produced, but also on their hatchability. Some of the vitamin fed passes into the egg yolk and is found in the chick at hatching. Within limits, the level of intake governs the amount thus stored. The requirement of the vitamin for egg production is much larger than for the growth of the chick. Murphy and associates<sup>2</sup> found that the inclusion of 78 U.S.P. units of vitamin D as cod-liver oil per 100 g. of feed was adequate for egg production and hatchability. The addition of the vitamin to the ration is unnecessary when the birds are given access to range, and thus to sunshine, throughout the laying period.

**295. Other Minerals.**—The iron requirement of the laying hen is very large in proportion to her maintenance need, as is evident from the fact that the average egg contains 1.1 mg. of this element. There have been reports of anemia in the laying hen, but other observations have been to the contrary. The evidence is conflicting as to whether commonly fed rations are benefited by iron supplements.

The egg is rich in several other mineral elements, but there is no evidence that any of them, aside from those supplied by common salt, are ever lacking in the commonly fed rations. Many studies have been made of the influence of added iodine on egg production and hatchability, with generally negative results, though the content of this element in the egg may be markedly increased.

**296. Vitamin E.**—Both the hen and the cock must have vitamin E in the diet, or reproduction fails. Unless the hen ingests the vitamin and puts it in her eggs, they fail to hatch, because the fertilized embryo dies. There is a degeneration of the testes of the male even as occurs in the mammal. The necessity of the

<sup>1</sup> MILLER, M. WAYNE, and G. E. BEARSE, Phosphorus requirements of laying hens, *Wash. Agr. Expt. Sta. Bull.* 306, 1934.

<sup>2</sup> MURPHY, R. R., J. E. HUNTER, and H. C. KNANDEL, The vitamin D requirements of growing chicks and laying hens, *Pa. Agr. Expt. Sta. Bull.* 334, 1936.

vitamin for hatchability was discovered by Card<sup>1</sup> of the University of Illinois and the specific pathological changes were described by his colleague, Adamstone.<sup>2</sup> During the first three days of embryonic development, the growth is retarded but is otherwise normal. In the next few days, however, death of the embryo occurs. At the end of the fifth day a "lethal ring" is generally found completely encircling the embryo, which occludes the blood vessels and causes asphyxiation.

**297. Other Vitamins.**—Vitamin A is necessary for egg production and for the hatching of strong chicks. The amount of the vitamin stored in the newly hatched chick is dependent upon the amount fed the hen. Sherwood and Fraps<sup>3</sup> report that 240 Sherman-Munsel units of the vitamin daily, corresponding to 3 units per gram of feed, are sufficient for egg production itself, but, that to produce eggs rich in the vitamin, 600 units daily, or 7.5 per gram of feed, are required.

Norris and associates<sup>4</sup> found that 130 micrograms of flavin per 100 g. of feed are satisfactory for normal egg production and that 230 micrograms are needed to produce eggs that will hatch well. The amount of flavin found in the egg is governed by the hen's diet.

Since the content of the egg in vitamins A, D, and G (flavin) is influenced by their level in the feed, it is evident that the ration fed the hen has an important effect on the dietary value of eggs in human nutrition.

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<sup>1</sup> CARD, L. E., Vitamin E requirements of poultry, *Poultry Sci.*, **8**, 328-334, 1929.

<sup>2</sup> ADAMSTONE, F. B., The effects of vitamin-E deficiency on the development of the chick, *J. Morphology Physiol.*, **52**, 47-90, 1931.

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<sup>4</sup> NORRIS, L. C., H. S. WILGUS JR., A. T. RINGROSE, VICTOR HEIMAN, and G. F. HEUSER, The vitamin-G requirement of poultry, *Cornell Agr. Expt. Sta. Bull.* 660, 1936.

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## CHAPTER XV

### LACTATION

A 1200-lb. cow producing 10,000 lb. of milk in a year secretes in this milk approximately two and one-half times as much dry matter as is present in her entire body. There are records of cows which have produced in the year's milk over five times the organic matter of their own bodies, and of cows which over a life time have secreted organic matter equivalent to thirty-five times that present in their own tissues. While less subject to direct measurement, it is apparent that milk secretion in the sow nursing a large litter also represents a noteworthy physiological performance. The studies of Macy and coworkers<sup>1</sup> show that the human organism is capable of producing an astonishing output of milk. It is clear that the metabolism of lactation is tremendous. While this metabolism includes many processes such as the digestion, absorption, circulation, and mobilization of nutrients, it particularly involves the functioning of the mammary glands. As an introduction to a consideration of nutritional requirements for lactation an understanding of the physiological processes concerned is essential.

**298. The Mammary Glands.**—The glands usually occur in pairs, the number of pairs varying with the species. They are modified cutaneous glands which make their appearance early in embryonic life but reach their full development only after a normal parturition. So far as is known they have no function other than milk secretion; for they can be removed at any stage of the life cycle without any observable harmful effect from their absence. A diagram showing the structure of the functioning udder is presented in Fig. 31.

The glands are present in a rudimentary form at birth and undergo little development until puberty, at which time a marked growth occurs. Thereafter there are periodic changes which are correlated with the ovarian cycle. Histological studies show that

<sup>1</sup> MACY, ICIE G., HELEN A. HUNSCHER, EVA DONELSON, and BETTY NIMS, Human milk flow, *Am. J. Diseases Children*, **39**, 1186-1204, 1930.

at each oestrus there is some duct growth in the gland, and an occasional secreting cell may be formed, which explains the fact that a watery secretion has been obtained before pregnancy. With the onset of gestation, there is a large increase in growth which involves the production of ducts, alveoli, and secreting cells. This growth occurs primarily during the first part of pregnancy. A secretory activity develops which results in an

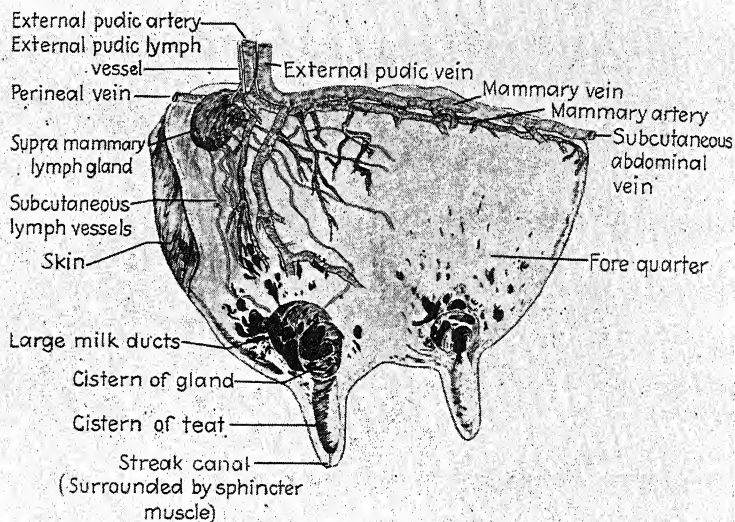


FIG. 31.—Cross section of functioning udder. (Furnished through the courtesy of C. W. Turner, University of Missouri.)

accumulation in the gland of products making up the colostrum. With the withdrawal of the secretion following parturition, its quantity gradually rises for a period which varies in different species, and then gradually falls until lactation ceases. As cessation occurs, the gland shrinks enormously, owing to a decrease in size of the alveoli and ducts, and remains quiescent until another pregnancy starts a renewed growth and secretion.

**299. Hormonal Control.**—The physiological mechanism controlling these various events is incompletely understood, but it is recognized that the activities of the ovary, uterus, and mammary gland are interrelated. It is now accepted that the essential stimulus for the growth and functioning of the gland is hormonal rather than nervous, and the present evidence indicates that at

least three hormones are concerned. Each one has a specific function, and the three act in a definite sequence. *Oestrone* (theelin), a hormone from the Graafian follicle, first causes a duct development. Next *progesterone* from the *corpus luteum* is responsible for the growth of the alveoli. Finally *prolactin* (galactin) from the pituitary gland brings about the secretory activity. As worked out primarily in the rabbit, it seems to be agreed that this represents the general picture. There is much uncertainty and lack of agreement regarding some of the details. There are doubtless some variations among the different species with respect to the relative amounts of the three hormones required to produce the effect and perhaps also as regards their mode of action.

The pituitary hormone is essential for the continuance of the secretory activity, but it is not primarily responsible for the

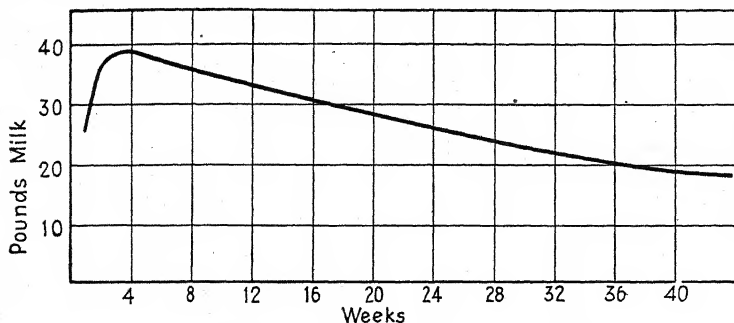


FIG. 32.—Lactation curve of the cow (not pregnant).

changes in the volume of secretion that occur with advancing lactation. The gradual decline in activity which occurs is due probably to a natural aging of the gland. The secreting cells which were built up during the previous pregnancy gradually cease to function, and little or no new growth takes place. For a detailed statement of the present ideas with respect to the role of the hormones in the development and functioning of the mammary gland, the student is referred to the publications of Turner<sup>1</sup> and of Asdell and associates.<sup>2</sup>

<sup>1</sup> TURNER, C. W., The mammary glands. Sex and internal secretions, pp. 544-583, Williams & Wilkins Company, Baltimore, 1932; The causes of the growth and function of the udder of cattle, *Mo. Agr. Expt. Sta. Bull.* 339, 1934.

<sup>2</sup> ASDELL, S. A., H. J. BROOKS, G. W. SALISBURY and H. R. SEIDENSTEIN, Experiments in the physiology of mammary development and lactation, *Cornell Agr. Expt. Sta. Mem.* 198, 1936.

**300. The Course of Milk Secretion.**—The normal lactation curve for the cow is shown in Fig. 32. The time involved in reaching the peak depends upon inherited factors and upon the condition of the cow prior to calving and how she is fed and managed thereafter. The rise in secretion following parturition does not run parallel with increased food intakes, and it extends over a much longer time than can be accounted for as the recovery period from the strain of calving. It may be caused by the gradual removal of the accumulated products enabling the cells to reach their maximum rate. However, the current nutrition of the animals is a secondary factor in milk secretion at the start of the lactation period, because the impulse to secrete is so strong at this time that the animal readily draws on her own reserves. Her previous nutrition which determines the status of her reserves at calving is thus concerned.

Following the peak, there is a regular decline in yield such that the curve is of the descending exponential type, each month's yield being a constant percentage of that of the preceding month. *Persistency* is the term used to denote the measure of this rate of decline, which varies with the individual and in different lactations, and which is accelerated at the twenty-second week after a new conception. The onset of a new pregnancy is thus the determining factor in the length of the lactation of the cow, both because of this accelerated decline and because of the necessity of giving her a rest period before another lactation. The fact that many cows will continue to secrete some milk right up to parturition, if milked regularly, shows that there is no physiological mechanism for absolutely stopping the process before this event. Cows which remain unbred may continue to secrete milk at a decreasing rate for two or three years or even longer.

Failure to remove the milk regularly and completely from the gland lessens its activity and brings about cessation, a fact which finds practical application in the drying off of animals. The onset of a new pregnancy during lactation results, after a period, in a more rapid decline in the secretion than otherwise occurs. In animals nursing young, lactation is usually artificially terminated at weaning, since failure to remove the secretion stops the process. Underfeeding during the declining period of secretion has an immediate effect in lowering the output, in contrast to its lesser



influence at the start of lactation, but no system of feeding will counteract in any way the normal decline.

The lactation curve of the goat is similar to that of the cow and milk secretion studies with sheep indicate a similar one for this species also. In the case of women, however, the peak is reached much later, or there is a plateau instead of a peak. The increase after parturition may continue to the twenty-eighth week, and the secretion may persist at or near its maximum level to the fortieth week.

With lactations of substantially equal length, the yield of the cow increases for the first four or five, on the average. The

TABLE XXIX.—PERCENTAGE COMPOSITION OF MILK OF DIFFERENT SPECIES<sup>1</sup>

Species	Water	Protein	Fat	Lactose	Ash	Calcium	Phosphorus	Calories	Authority
Cow.....	87.2	3.5	3.7	4.9	0.72	0.121	0.095	74.3	Trautmann and Kirchhof
Sheep....	82.7	5.5	6.4	4.7	0.92	0.201	0.168	110.9	
Goat....	86.5	3.6	4.0	5.1	0.81	0.131	0.104	79.5	Linton
Mare....	89.0	2.7	1.6	6.1	0.51	.....	.....	54.9	
Sow.....	82.0	6.2	6.8	...	0.96	0.252	0.151	113.6	Hughes and Hart
Woman..	87.5	1.0	4.4	7.0	0.21	0.035	0.013	70.0	Macy et al.

<sup>1</sup> The data for the cow and goat were computed by the writer. The publications from which the other data were taken are cited at the end of the chapter. The data for human milk are expressed as grams per 100 cc. With the exception of this milk, the data for calorie content were calculated by the writer.

growth of the animal as a whole is a factor during the first three lactations, and there is markedly greater hypertrophy of the gland during the second pregnancy than during the first, with smaller increases in succeeding pregnancies. Persistency decreases in succeeding lactations. Since this is true it is apparent that the higher yields which are obtained in succeeding lactations must be due to a greater secretion during the first part of the lactation. Expressed another way, it may be said that the level, at which secretion begins, increases to maturity but that the total yield for the lactation is not proportional to this rise because of a declining persistency factor.

The longer the dry period the greater the persistency in the next lactation. Shortening the dry period before a second lacta-

tion lowers yield to a much greater extent than shortening it before a later lactation. This is readily explainable on the basis of the greater growth of body and gland that takes place before the second lactation than later. The importance of an adequate dry period to build up nutrient reserves is well understood.

**301. Milk of Different Species.**—The chemical composition of the milk of the various species is presented in Table XXIX. It should be emphasized that the figures for individual animals may vary widely from these average values. This is certainly true for cows of different breeds and for individuals within the breed as is discussed later (Sec. 313). Doubtless the variations have become greatly accentuated in this species as a result of breeding operations, but they must be expected to occur to a certain extent in all species. The data presented in the table are useful, nevertheless, to indicate the nutrients involved in milk secretion and to bring out certain differences which exist among the species. It is noted that the milk of the sow and ewe contains considerably more dry matter than the milk of any of the other species and that this is reflected in a higher energy value and ash content. Particularly noteworthy is the much higher content of calcium and phosphorus. Clearly per unit of product, the nutritive requirements for milk secretion in the sow and ewe are markedly greater than in the cow, goat, or mare. It is also interesting to note that the least variable constituent for all species is lactose. The same is true among individuals and also for different samples from the same individual, as is brought out in later discussions for the cow.

#### THE SECRETION OF THE MILK CONSTITUENTS

**302. The Composition of Blood and Milk.**—Most of the organic constituents of milk arise from specific synthetic processes of the mammary gland, representing products which are not found elsewhere in nature. As an aid to a consideration of the physiology involved and the chemical changes which take place there are presented side by side, in Table XXX, data as to the composition of the milk and of the blood plasma of the cow. The different milk constituents are listed opposite the respective blood constituents from which they are believed to be derived. Several other constituents not shown in the table are present in both fluids, such as urea and other nonprotein-nitrogen compounds,

pigments, free cholesterol, and various minerals not listed. A study of the figures in the table makes it evident that milk has a very different composition, both quantitatively and qualitatively, from the blood plasma from which it is made; but the two fluids have one characteristic in common—they are isotonic.

Certain milk constituents, including lactose, casein, and some of the fatty acids are clearly synthetic products of the gland, while others such as the minerals and urea, pass directly from the blood stream. It is probable that the albumin and globulin of milk, and its cholesterol also, arise directly from the blood. It may be debated as to what ones of these constituents, which come in

TABLE XXX.—COMPARATIVE COMPOSITION OF BLOOD PLASMA AND MILK OF THE COW

Blood plasma		Milk	
Composition	Per cent	Composition	Per cent
Water.....	91.0	Water.....	87.0
Glucose.....	0.05	Lactose.....	4.9
Serum albumin.....	3.20	Lactalbumin.....	0.52
Serum globulin.....	4.40	Lactoglobulin.....	0.05
Amino acids.....	0.003	Casein.....	2.9
Neutral fat.....	0.20	Neutral fat.....	3.7
Phospholipids.....	0.15	Phospholipids.....	0.04
Cholesterol ester.....	0.09	Cholesterol ester.....	trace?
Calcium.....	0.009	Calcium.....	0.12
Phosphorus.....	0.011	Phosphorus.....	0.10
Sodium.....	0.34	Sodium.....	0.05
Potassium.....	0.03	Potassium.....	0.15
Chlorine.....	0.35	Chlorine.....	0.11
Citric acid.....	trace	Citric acid.....	0.20

unchanged, are there by design and what ones represent accidental though constant filtrations. Urea would seem to be in the latter class, although it may be in part a product of gland metabolism.

The milk of the individual cow tends to be of constant composition, but there are periodic fluctuations, notably of fat, and there are characteristic changes over the course of the lactation. With the normal decline in yield the percentage of fat rises, and so does the protein to a lesser degree. In contrast, the lactose declines

slightly, and, for the maintenance of osmotic relations, its decline is balanced by a rise in chlorides. These same changes tend to occur when the yield is subjected to an abnormal drop as the result of sickness, "off-feed," or other disturbing factors. Overman and coworkers<sup>1</sup> have published very extensive data on the composition of the milk of different breeds of cows over the lactation period. Their study shows that as the percentage of fat increases, the percentage of protein, ash, and total solids tends to increase and that the percentage of lactose tends to decrease. The composition of human milk is apparently more variable than that of the cow.

**303. Mechanism of Milk Secretion.**—A complex series of reactions are involved in the secretion process, *viz.*, the passage of blood constituents into the cells, the synthesis of secretory granules and their later breakdown, the transfer of water to dissolve the granules, and the passage of the product into the ducts. Many explanations have been proposed for the physical changes concerned but none of them are entirely satisfactory. A discussion of milk secretion in terms of physicochemical phenomena is given by Blackwood and Stirling.<sup>2</sup>

Histological evidence has disproved the earlier view that the cells are completely destroyed in the process of secretion, the granules forming the lactose and fat, and the nuclei forming the protein of milk. At present some workers adhere to the theory of Heidenhain that there is a partial disintegration of the cell whereby the constituents escape, while others believe that a true secretion is involved. There is histological evidence for both explanations. The most rapid rate of secretion occurs after milking, and there is a gradual decline with time until the product is again removed. This decline is due to the increasing pressure in the udder, as indicated by the work of Swett and associates<sup>3</sup> and by later investigations. This declining rate of secretion

<sup>1</sup> OVERMAN, O. R., F. P. SANMANN, and K. E. WRIGHT, Studies of the composition of milk, *Ill. Agr. Expt. Sta. Bull.* 325, 1929.

<sup>2</sup> BLACKWOOD, JANET HENDERSON and JOHN DEMPSTER STIRLING, The absorption of milk precursors by the mammary gland. V. Physicochemical aspects of milk secretion, *Biochem. J.*, 26, 1127-1137, 1932.

<sup>3</sup> SWETT, W. W., FRED W. MILLER, R. R. GRAVES, and G. T. CREECH, Quality, size, capacity, gross anatomy, and histology of cow udders in relation to milk production, *J. Agr. Research*, 45, 577-607, 1932.

with the accumulation of milk in the udder is the probable explanation of the fact that larger daily yields are obtained from high producers by more frequent milking. Conversely, milk pressure is the responsible agent for stopping the secretion when milk is left in the udder as a means of drying off the cow.

When the milking act is initiated, a small quantity of milk can be immediately removed, then there is a lag period followed by a large inflow of milk into the cistern of the udder. One says that the cow "has let down" her milk. This is believed to be a reflex action as a result of the stimulation of the teat in the act of milking or suckling. An erection of the gland results, either from the inflow of venous blood or from muscle contraction, whereby the milk is forced out of the alveoli and ducts. The evidence for this explanation is convincingly presented by Hammond<sup>1</sup> in an article which discusses various relations of pressure to milk secretion.

As previous discussion indicates, milk secretion is a continuous process, contrary to the former view that most of the milk is secreted at the time of milking. It was formerly thought that the udder of the high-producing cow could hold only a fraction of the milk obtained at a given milking. Experiments by Swett and associates<sup>2</sup> and by others, in which udders have been milked after removal from the body, have disproved this idea.

**304. Blood Changes in Relation to Secretion.**—A useful method for the study of milk secretion is the analysis of the blood before and after passing the gland. Data obtained in this way have provided important information as to the blood precursors of the milk constituents, but they cannot establish quantitative relations between blood changes and the constituents secreted, because no one has been able to measure the blood volume passing through the gland. In view of the many outgoing paths, including the lymphatics, such a measure appears impossible. Further, milk secretion is very easily disturbed by external conditions, and, therefore, it is very doubtful whether quantitative data of value could be obtained in an animal disturbed by drawing of blood samples and by the measure of the volume flowing through the gland.

<sup>1</sup> HAMMOND, JOHN, The physiology of milk and butter fat secretion, *Vet. Record*, 16, 519-537, 1936.

<sup>2</sup> SWETT, MILLER, GRAVES, and CREECH, *loc. cit.*

**305. The Secretion of Lactose.**—It is generally accepted that glucose is the blood precursor of lactose. Kaufmann and Magne<sup>1</sup> took samples simultaneously from the jugular and mammary veins of a milking cow and found that the mammary blood contained 18 per cent less glucose, whereas similar samples from a dry cow showed no difference. Considering the jugular blood as representative of the supply of the gland, they concluded that the lactose of milk was made from the glucose of the blood. Somewhat later Foa obtained, by perfusion experiments, specific evidence that the gland can use glucose to make lactose. The finding of Kaufmann and Magne has been repeatedly confirmed, notably by Blackwood and Stirling<sup>2</sup> who refined the procedure by comparing arterial blood with mammary-vein blood and by determining the changes in true sugar as distinguished from total reducing substances.

It has been assumed that the transformation of glucose into lactose involves first the formation of galactose from glucose and then the union of galactose with another molecule of glucose. But, from the standpoint of the chemist, the stereochemical transformation of glucose to galactose is difficult to understand. Many attempts to find, in glandular tissue, enzymes that would cause this change and combine the two sugars have given negative results. Michlin and Lewitow<sup>3</sup> have reported the synthesis of lactose from glucose and galactose under the action of tissue from an active gland. Grant<sup>4</sup> obtained milk sugar from glucose in the presence of tissue slices from the active gland, but little or no lactose was produced from galactose, mannose, or fructose. In a later study he found that the synthesis of lactose was no more rapid from a mixture of glucose and galactose than from glucose alone.

<sup>1</sup> KAUFMANN, M., and H. MAGNE, Sur la consommation du glucose du sang par le tissu de la glande mammaire, *Compt. rend. acad. sci.*, **143**, 779-782, 1906.

<sup>2</sup> BLACKWOOD, JANET HENDERSON, and JOHN DEMPSTER STIRLING, The absorption of milk precursors by the mammary gland. II. The relation of blood-sugar absorption to lactose secretion, *Biochem. J.*, **26**, 362-368, 1932.

<sup>3</sup> MICHLIN, M., and M. LEWITOW, Synthese von lactose in der milchdrüse, *Biochem. Z.*, **271**, 448-54, 1934.

<sup>4</sup> GRANT, GORDON A., The metabolism of galactose. II. The synthesis of lactose by slices of active mammary gland in vitro, *Biochem. J.*, **29**, 1905-1909, 1935.

During lactation the glucose content of the blood is lower than when the gland is not functioning. This has raised the question as to whether the glucose level of the blood is a controlling factor in milk secretion. Injections of lactose, glucose, and other sugars have not furnished any consistent evidence that the volume of secretion or its percentage of lactose can be increased thereby. Several investigators have shown that the lowering of blood sugar which results from the injection of insulin or phlorizin is accompanied by a lower milk yield. This does not prove that the change in milk was the direct result of the change in blood sugar. A decrease in lactose percentage has been reported from some of these experiments and interpreted to be a direct result of a lowering of its precursor. Other explanations are possible. At the present time the various experimental data furnish no reliable evidence that either the amount of milk secretion or its lactose content is influenced by the level of blood glucose. There is no evidence that the lactose percentage is influenced by diet.

**306. The Secretion of Fat.**—The lipids of milk consist mostly of neutral fats or triglycerides, with small amounts of phospholipid, cholesterol ester, and free cholesterol. Differing from fat found in other animal products or in plants, milk fat contains a considerable amount of glycerides of short-chain fatty acids (Table VII). The formation of these acids represents a synthetic action by the gland. The distribution of the fatty acids in milk can be modified somewhat by the nature of the diet even as is the case for depot fat, but not sufficiently to alter the specific character of milk fat. While either fat or carbohydrate, and presumably protein also, may serve as the food source of milk fat, fat itself is apparently the preferred source when there is an adequate supply in the diet. Carbohydrates and protein must first be changed into fat before they are used by the gland, for the milk fat is made from blood lipids.

The question as to what lipid fraction in the blood is used by the gland has long been a subject of study. In the blood plasma of the lactating cow about 50 per cent of the fatty acids are present as neutral fats, the balance being about equally divided between phospholipid and cholesterol ester. In 1911 Foa came to the conclusion, based on rather meager evidence from perfusion studies, that milk fat was synthesized from neutral fats in the blood. In 1919, Meigs and associates obtained evidence that the

lipid phosphorus was lower in the mammary-vein blood than in the jugular-vein blood and suggested that phospholipids were the precursor of milk fat. This idea was in accord with the special role attributed to phospholipids in the transport of fat, and it became rather generally accepted. Recent, and more extensive, experiments by several workers have failed to confirm the findings of Meigs. In a carefully conducted study with the lactating goat, Lintzel<sup>1</sup> found that neither the phospholipid fatty acids nor the cholesterol-ester fatty acids were lowered in the blood in its passage through the gland. Since there was a lowering of the total fatty acids, calculations indicated that neutral fat was taken up by the gland. Similar evidence in the case of the lactating cow has been obtained by Maynard and associates.<sup>2</sup> It thus appears at the present time that triglycerides furnish the fatty acids from which milk is synthesized, in accord with the original idea of Foa, but direct proof for this view remains to be shown.

While depot fats can be called upon to furnish the precursor of milk fat, it is possible that with an adequate supply in the ration they play little role in the process. Maynard, McCay, and Madsen<sup>3</sup> have shown that food fat appears in the milk with surprising speed. At the start of lactation the cow unquestionably makes use of her reserves for milk production but whether these reserves are used specifically to make fat or to furnish energy cannot be stated. Experiments by Petersen and associates<sup>4</sup> indicate that in the process of milk secretion, a fat intermediate in character between body fat and milk fat is first deposited in the glandular tissue and then further modified to form the milk fat itself. While it is evident that the lower-carbon fatty acids of milk are manufactured in the gland, since they do not occur in the blood stream, the mechanism involved is a mystery.

<sup>1</sup> LINTZEL, WOLFGANG, Untersuchungen über den Chemismus der MilCHFETTBildung in Abhängigkeit von der Fütterung, *Z. Zucht. Riehe B, Tierzucht. u. Zuchtungsbiol.*, **29**, 219-242, 1934.

<sup>2</sup> MAYNARD, L. A., A. Z. HODSON, G. H. ELLIS, and C. M. MCCAY, The blood precursor of milk fat, *J. Biol. Chem. Proc.*, **119**, LXVI-LXVII, 1937.

<sup>3</sup> MAYNARD, L. A., C. M. MCCAY, and L. L. MADSEN, The influence of food fat of varying degrees of unsaturation upon blood lipids and milk fat, *J. Dairy Sci.*, **19**, 49-53, 1936.

<sup>4</sup> PETERSEN, W. E., L. S. PALMER, and C. H. ECKLES, The synthesis and secretion of milk fat. III. A study of the activity of the perfused surviving gland. With special reference to the fat, *Am. J. Physiol.*, **90**, 592-599. 1929.



**307. Variation in the Secretion of Fat.**—Fat is the most variable constituent of milk. In addition to its variation among breeds and individuals, its percentage in the milk of a given animal varies from milking to milking, from quarter to quarter, and increases progressively during the milking process. In a study with a Guernsey cow, Van Slyke found that the first fraction drawn, consisting of about 18 per cent of the whole, contained only 1 per cent of fat, whereas the final fraction of 30 per cent contained 10 per cent of fat. It is very difficult to find an explanation for these large variations. Hammond<sup>1</sup> has set forth important evidence that differences in fat percentage are caused by milk pressure, through the inhibition of the actual secretory process and through its effect on the ease with which the fat globules pass down the ducts to the cistern. He states that changes in the chemical composition of milk occur as the rate of secretion varies.

Periodic changes in fat percentage are inversely correlated with milk yield. A lowering of yield which results from an abrupt change in conditions, such as weather, surroundings, or even a change in the milker, is frequently accompanied by a rise in fat content. The sharply lowered yield which results during a period of "off feed" is generally accompanied by a rise in fat percentage. This inverse relationship is also shown in the course of the lactation, for as the decline in yield progresses following the peak the percentage of fat tends to rise.

There is a marked seasonal variation in the percentage of fat, the maximum occurring in winter and the minimum, in summer. That environmental temperature is the primary cause of this variation is indicated by controlled studies in which it has been shown that within certain limits fat percentage increases regularly with drop in temperature. From one lactation to another there is no consistent change in fat percentage. While an increase in milk yield may be expected during the first four or five lactations, a cow which has a low fat test as a heifer will not better it materially in succeeding years.

Maynard and coworkers<sup>2</sup> have made extensive studies of the possible relation between the level of blood lipids and the milk-

<sup>1</sup> HAMMOND, *loc. cit.*

<sup>2</sup> PORCHER, CH., and L. MAYNARD, La graisse du sang et la graisse du lait pendant la lactation, *Le lait*, 10, 601-613; 765-782, 1930. MAYNARD, L. A.,

fat percentage. Their comparative studies of the blood and milk from milking to milking, from day to day, and from week to week have revealed no changes in the blood which were correlated in any way with the large variations which frequently occurred in milk-fat percentage. A ration which resulted in a marked decrease in the concentration of the blood lipids did not produce any corresponding changes in the milk fat. Their studies show-

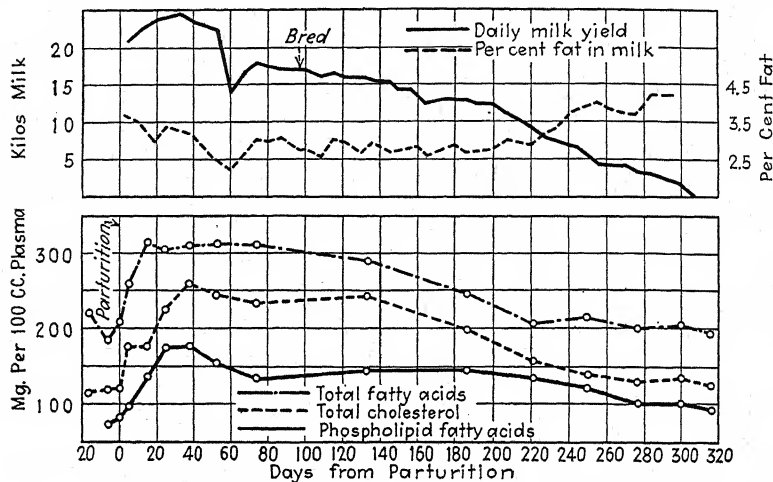


FIG. 33.—The blood lipid levels during the lactation cycle in relation to the changes in milk yield and in fat percentage. (Maynard, Harrison, and McCay.)

ing that, over the lactation cycle as a whole, the level of blood lipids tends to follow the course of milk yield rather than the fat percentage are illustrated in Fig. 33. Specifically, it may be noted that, though the fat percentage rises markedly toward the end of the lactation, the blood lipid levels tend to fall rather than otherwise. While a declining food and fat intake undoubtedly operates to cause a drop in blood lipid level, the investigators found that there was a response in blood lipids with change in milk yield when the food intake was held constant.

E. S. HARRISON, and C. M. MCCAY, The changes in the total fatty acids, phospholipid fatty acids, and cholesterol of the blood during the lactation cycle, *J. Biol. Chem.*, **92**, 263-272, 1931. MAYNARD, L. A., and C. M. MCCAY, The influence of a low-fat diet upon fat metabolism during lactation, *J. Nutrition*, **2**, 67-81, 1929; The influence of different levels of fat intake upon milk secretion, *Cornell Agr. Expt. Sta. Bull.* 543, 1932.

Contrary to the above findings, Leroy and Marcq<sup>1</sup> have concluded that there is a correlation between blood lipid level and the fat test.

Though the distribution of the fatty acids in milk fat can be modified by diet, there is no reliable evidence that the fat percentage can be altered significantly over any extended period by any of the commonly used feeds. The feeding of butter to a cow has been shown to raise the fat percentage, at least over a brief period. The explanation may lie in the fact that the gland is thus presented with the same fatty-acid mixture as is found in its secretion. There are several reports of positive effects with various feeds of vegetable origin, and there are an equal number of reports to the contrary with the same feeds. Failure to take adequate account of the many nondietary factors which cause the fat percentage to vary is doubtless responsible for the contradictory results obtained. Among the many feeds which have been claimed by various workers to raise the fat percentage, press cakes high in fat of copra, the palm kernel, and the babassu nut are the only ones for which the evidence seems at all consistent. The increases reported are in general small, and they have not been found to occur in all the animals studied or with all rations in which the feeds were included. They may, however, represent a real effect. If this is true, the fact that these feeds are rather unique in containing considerable amounts of some of the lower fatty acids which are found in milk fat may be responsible.

While repeated efforts to increase the fat percentage in milk have generally failed, cod-liver oil exerts a marked effect in lowering it. This peculiar response was first observed by Golding and associates<sup>2</sup> in connection with studies of the influence of the oil on the vitamin content of the milk. It has since been confirmed in several laboratories. Feeding as small amounts as 2 oz. per day has caused a lowering, but larger and more consistent effects are observed with intakes of 4 to 6 oz. A 30 per cent decrease in the fat level has been noted. The specific factor in the oil which is responsible is found in the saponifiable fraction, but it has not

<sup>1</sup> LEROY, ANDRÉ-M., and J. MARCQ, Le taux butyreux sanguin des reproducteurs bovins et ses rapports éventuels avec la valeur d'élevage, *Le lait*, 11, 12-21, 144-155, 234-244, 359-369, 1931.

<sup>2</sup> GOLDING, JOHN, KATHARINE MARJORIE SOAMES, and SYLVESTER SOLOMON ZILVA, The influence of the cow's diet on the fat-soluble vitamins of winter milk, *Biochem. J.*, 20, 1306-1319, 1926.

been identified. There may be some relation between the factor here involved and the one which is responsible for muscle injury in Herbivora (Sec. 75) since both are found in the saponifiable fraction. Limited study suggests that this property of lowering the fat percentage is not shared by fish oils generally. Clearly vegetable oils do not exert the effect.

**308. The Secretion of Protein.**—The very small amount of globulin which is present in milk is identical with the globulin of blood plasma and is considered by most workers to represent merely a filtration product. Many workers also believe that lactalbumin is identical with the albumin of blood and that its origin in the milk is similar to that of the globulin. Casein, which makes up nearly 90 per cent of the milk protein, is clearly a synthetic product. Studies first carried out by Cary<sup>1</sup> and later confirmed by other workers, showing that the amino-acid content of the blood plasma suffers a drop in passing the gland, furnish evidence that casein is made from amino acids, as is true for body proteins in general. There is no knowledge regarding the specific chemical changes which are involved in its synthesis in the gland. The small amounts of urea and other nonprotein-nitrogen compounds which are found in milk are probably filtrates from the blood, though they may be catabolic products of glandular activity.

**309. The Secretion of the Mineral Elements.**—The data presented in Table XXX show that milk contains the same minerals as are present in blood plasma but that the quantitative relations are very different. How the gland selectively secretes its minerals presents a physicochemical problem which remains largely unexplained.

For a given animal, the mineral constituents normally show little variation, either periodically or over the lactation as a whole. This is brought out by the extensive mineral-balance studies of Forbes and coworkers.<sup>2</sup> Neither diet nor blood changes have

<sup>1</sup> CARY, C. A., Amino acids of the blood as the precursors of milk proteins, *J. Biol. Chem.*, **43**, 477-489, 1920.

<sup>2</sup> FORBES, ERNEST B., ALEX BLACK, WINFRED W. BRAMAN, DONALD E. H. FREAR, ORME J. KAHLENBERG, FRANK J. McCLURE, RAYMOND W. SWIFT, and LE ROY VORIS, The mineral requirements of milk production: the annual cycle of mineral and nitrogen metabolism of the milch cow as affected by alfalfa hay, timothy hay, bone flour and ground limestone, *Pa. Agr. Expt. Sta. Tech. Bull.* 319, 1935.

any influence on the calcium, phosphorus, or iron content of milk. The small amount of iodine which is present in milk can be increased, however, by adding an iodine compound to the ration (Sec. 128). Citrates of the electropositive minerals make up 30 per cent of the milk ash. The role of citric acid in milk is only partially understood, and nothing is known regarding its secretion.

**310. Pigments.**—Milk contains both fat-soluble and water-soluble pigments. Of the fat-soluble, carotenoid group of pigments, which are synthesized by plants but not by animals, *carotene* is the principal one found in the milk of the cow. Chlorophyll is destroyed in the digestive tract, and this is presumably true to a certain extent also for xanthophyll, only a small amount of which gets into the milk. The occurrence of carotene in milk is limited primarily to the bovine species. The milk of the sheep, goat, and camel has little or none, and women's milk is nearly colorless. The reason for these breed and species differences is not known, but when there is no pigment in the milk the blood plasma is also free. The principal water-soluble pigment of milk is *lactoflavin* (Sec. 166), formerly known as *lactochrome*. Since carotene is the precursor of vitamin A and since lactoflavin is also a vitamin, the factors which govern their secretion in milk are best discussed later where the vitamin requirements for lactation are considered.

**311. The Nature of Colostrum.**—The first product from the mammary gland following parturition, the colostrum, is richer in total solids and total ash and much richer in protein, but lower in lactose, than normal milk. The proteins, which make up approximately 17 per cent of the product in the case of the cow, consist principally of globulin and albumin, in marked contrast to their very low content in normal milk. Colostrum is rich in antibodies which apparently immunize the newborn temporarily against certain diseases, and it has a laxative action. In the course of the first few days of the lactation, it gradually assumes the composition of normal milk.

Formerly it was considered that colostrum was a special secretion of parturition designed as a more suitable food for the newborn than milk itself, but it is now recognized that this special product can be obtained weeks prior to parturition by manipulating the gland and that secretory activity can be stimulated at this time. As a result the colostrum gradually assumes the character

of normal milk, even as it does after parturition, and if the milking is continued uninterrupted through parturition, no further change in the character of the secretion occurs. While accepting these facts, many still believe that colostrum is a special product resulting from the secretion of large amounts of globulin around the fourth and fifth month of pregnancy, and that this globulin has the specific function of carrying the antibodies. There is considerable support, however, for the view put forward by Porcher<sup>1</sup> that colostrum is merely a normal milk which has been modified by the resorption of some of its constituents, notably water, lactose, and salts, as a result of its not being removed from the gland. He showed that a similar product could be obtained by allowing the milk to remain in the gland for an extended period instead of removing it regularly, and he produced other experimental evidence for his explanation.

It is recognized as desirable that the newborn receive the colostrum because of its laxative and immunizing effects. A recently discovered further reason is that colostrum contains many times as much vitamin A as the normal milk which is obtained later. This finding by Dann<sup>2</sup> has been confirmed by other workers. Vitamins C and D have also been reported to be somewhat more concentrated in the colostrum than in the later secretion.

**312. Abnormal Milk Constituents.**—It has been mentioned that some of the normal constituents of milk are apparently merely filtration products. Among these there are substances, such as urea, which represent useless products and which apparently pass into the milk in small amounts instead of being excreted entirely through the usual channels, because the membrane is not a perfect barrier. This fact raises the question as to the extent to which such substances as drugs and other non-nutritive or toxic substances may pass into the milk in health and in disease. This subject has been discussed very comprehensively by Kolda<sup>3</sup> with the general conclusion that the healthy gland is

<sup>1</sup> PORCHER, CH., La retention lactée, *Ann. fals.*, **12**, 329-343, 1919.

<sup>2</sup> DANN, WILLIAM J., The transmission of vitamin A from parents to young mammals. II. The carotene and vitamin A content of cow's colostrum, *Biochem. J.*, **27**, 1998-2005, 1933.

<sup>3</sup> KOLDA, J., Du passage des substances médicamenteuses dans le lait, *Le lait*, **6**, 12-24, 88-102, 180-194, 269-287, 1926.

highly protective against the passage of foreign substances in harmful concentrations. Neither heavy metals, such as mercury and arsenic, nor volatile organic substances, such as alcohol, ether, and chloroform, pass into the milk in toxic amounts. The same was found true for salicylic acid, aspirin and related compounds, and for various alkaloids such as morphine, atropine, and quinine. It is recognized, however, that certain essential oils may pass into milk to the extent of causing an odor and taste, and that the poisonous principles of certain plants, such as white snake root and rayless golden rod, may be secreted in sufficient amounts to render the milk harmful.

In diseases of the udder, particularly those characterized by inflammation, the membrane becomes much more permeable. The milk itself changes in the direction of the composition of blood with a resulting increase in protein and salts and a decrease in lactose. The protein fraction contains more albumin and less casein. With a more permeable gland, the danger from the entrance of harmful foreign substances is greatly increased. In diseases not affecting the udder, the primary effect is on yield of milk rather than upon its composition.

### THE ENERGY REQUIREMENT

In addition to her maintenance requirement, the lactating animal must receive sufficient nutrients to supply those secreted in her milk and to cover the wastage involved in the process. It is obvious that a separate requirement can be stated for lactation only for those animals for which the current milk yield and its composition are known, *i.e.*, for those used in commercial milk production. Thus we have specific knowledge of the nutritive needs for milk secretion only in the case of the dairy cow and goat. The following discussions deal principally with the cow.

**313. Variations in Milk Composition.**—While the milk of the individual cow tends to remain constant in composition, aside from fluctuations and cyclic changes in fat content, there are wide differences among individuals within a given breed as well as among the breeds themselves. This is shown by some data taken from the studies of Overman and Gaines<sup>1</sup> and presented in Table XXXI. The complete data given by these workers cover

<sup>1</sup> OVERMAN, O. R., and W. L. GAINES, Milk-energy formulas for various breeds of cattle, *J. Agr. Research*, **46**, 1109-1120, 1933.

an even wider range of fat percentage. The figures presented serve to illustrate the regular increases in protein and energy content that occur with a rising fat percentage and, in contrast, the nearly constant level of lactose that is maintained.

The data clearly show that the protein and energy requirements for milk production must be based on the composition of the

TABLE XXXI.—COMPOSITION OF HOLSTEIN AND JERSEY MILK AS RELATED TO ITS FAT CONTENT

Fat-percentage class	No. of records	Fat, %	Protein, %	Lactose, %	Ash, %	Water, %	Energy per kg. of milk, Cal.	Protein per Cal. mg.
<b>Holsteins:</b>								
2.60 to 2.79	8	2.709	3.055	5.000	0.656	88.58	616.4	49.6
2.80 to 2.99	25	2.890	3.012	4.928	0.666	88.51	628.6	47.9
3.00 to 3.19	40	3.104	3.169	4.846	0.672	88.21	654.1	48.4
3.20 to 3.39	47	3.289	3.276	4.884	0.671	87.88	678.6	48.3
3.40 to 3.59	42	3.488	3.388	4.853	0.688	87.58	702.9	48.2
3.60 to 3.79	35	3.674	3.361	4.866	0.672	87.43	718.5	46.8
3.80 to 3.99	29	3.874	3.502	4.863	0.681	87.08	745.2	47.0
4.00 to 4.19	11	4.078	3.734	4.858	0.694	86.64	777.2	48.0
4.20 to 4.39	13	4.313	3.967	4.722	0.697	86.30	806.4	49.2
<b>Jerseys:</b>								
3.80 to 3.99	8	3.910	3.623	4.860	0.683	86.92	755.1	48.0
4.00 to 4.19	5	4.074	3.302	4.866	0.660	87.10	752.2	43.9
4.20 to 4.39	11	4.292	3.695	5.126	0.697	86.19	806.1	45.8
4.40 to 4.59	18	4.493	3.648	5.058	0.691	86.11	819.3	44.5
4.60 to 4.79	16	4.668	3.556	5.059	0.681	86.03	830.2	42.8
4.80 to 4.99	20	4.881	3.715	5.058	0.693	85.65	859.3	43.2
5.00 to 5.19	19	5.091	3.896	4.919	0.694	85.40	883.1	44.1
5.20 to 5.39	26	5.295	3.859	5.026	0.698	85.12	904.7	42.7
5.40 to 5.59	16	5.479	3.891	4.986	0.693	84.95	921.7	42.2
5.60 to 5.79	16	5.707	4.171	4.963	0.743	84.42	959.7	43.5
5.80 to 5.99	12	5.892	4.288	4.929	0.732	84.16	981.4	43.7
6.00 to 6.19	8	6.081	4.264	4.764	0.726	84.17	990.9	43.0
6.20 to 6.39	5	6.230	4.010	4.982	0.713	84.06	999.2	40.1

milk. Since they increase with fat content, formulas can be devised for computing the protein and energy content from fat percentage. This is very helpful because it is a simple matter by the Babcock test to determine the fat percentage sufficiently frequently to provide the basis for arriving at the nutritive requirements, whereas the determination of protein and energy would be impracticable. The relations are somewhat different for the various breeds as is evident from the data for protein per Calorie given in the table. Overman and Gaines devised



formulas for various breeds for calculating the energy content from fat percentage. They concluded, however, that, for practical purposes, it was permissible to use a single formula, earlier proposed by Gaines,<sup>1</sup> which could be expressed in terms of 4 per cent milk as follows:

$$4\% \text{ milk} = 0.4M + 15F$$

$M$  is the weight of milk and  $F$  the weight of fat contained in it. The amount of 4 per cent milk thus obtained is multiplied by its calorific value (750 Cal. per kilogram) giving the energy content per kilogram of the original milk. A simplified procedure for applying the Gaines formula has been designed by Perkins.<sup>2</sup>

Overman and Gaines also give formulas for each breed for computing protein content from energy value. They use the basis of 50 mg. of protein per Calorie as an approximate relation applying to all breeds.

**314. Estimation of Energy Requirements from Metabolism Studies.**—A number of workers who have carried out energy-balance studies with dairy cows have estimated the energy requirements for milk production from data as to the energy content of the milk and data as to the percentage of feed energy recovered in its secretion. Kriss<sup>3</sup> has reviewed the different procedures used by these workers. As an example of the basic feature involved, the recently published method of Forbes and Kriss<sup>4</sup> may be outlined.

From a consideration of 3 complete energy balances by Kellner and 11 carried out at the Pennsylvania Institute of Animal Nutrition, it was found that the average utilization of metabolizable energy for milk production was 69.3 per cent. Forbes and

<sup>1</sup> GAINES, W. L., The energy basis of measuring milk yield in dairy cows, *Univ. of Ill., Agr. Expt. Sta. Bull.* 308, 1928.

<sup>2</sup> PERKINS, A. E., A simplified procedure for calculating weights of milk to their energy equivalent in milk of different fat content in accordance with the Gaines formula, *J. Dairy Sci.*, **20**, 129-132, 1937.

<sup>3</sup> KRISS, MAX, A comparison of feeding standards for dairy cows, with special reference to energy requirements, *J. Nutrition*, **4**, 141-161, 1931.

<sup>4</sup> FORBES, E. B., and MAX KRISS, The nutritive requirements of the dairy cow expressed in accord with a new method of application of the net-energy conception. *Proc. Am. Soc. Animal Production*, 1931, pp. 113-120; The nutritive requirements of the dairy cow expressed in accord with a new method of application of the net-energy conception: a correction, *ibid.*, 1932, pp. 344-345.

Kriss used this factor to compute the metabolizable energy requirement per pound of milk for milks of different energy contents. From a study of the data of 77 experiments in the respiration calorimeter, they found that, for the rations employed, the average metabolizable energy per pound of total digestible nutrients was 1.616 Therms. This provided a basis for translating their metabolizable energy requirements into total digestible nutrients. Thus, from a consideration of the relation between fat percentage and energy content in milk, they were able to express the requirements in terms of the commonly used method, *viz.*, total digestible nutrients per pound of milk for different fat percentages. For example, the requirement for 1 lb. of 3 per cent milk was stated as 0.251 lb. of total digestible nutrients, for 4 per cent milk, 0.30 lb., and the like. The writers recognized that their standards thus suggested might require revision after trial in practice, and they proposed them as an illustration of the method of estimation rather than as final recommendations.

### 315. Energy Requirements as Determined in Feeding Trials.—

The energy requirements for milk production which are specified in the commonly used standards have been derived principally from feeding trials. The general procedure here has been to record the feed intakes and production of groups of cows differently fed and to select as the requirements those intakes which were found to give the best production over extended periods and, at the same time, maintain the weight of the animal. By the subtraction of a maintenance requirement determined after the cows were dry, or upon other animals, the net intakes required for the milk produced were obtained. Most commonly the resulting data have been expressed as total digestible nutrients, based either upon digestion trials run as a part of the experiment or else calculated by the use of the average coefficients for digestibility (Sec. 190).

Haecker, who was the first to recognize that the food requirements must vary in accordance with the composition of the product, began in 1897 a series of investigations of nutrient requirements for milk production which extended over many years. His publication in 1914<sup>1</sup> set forth total-digestible-nutrient requirements in accordance with the milk produced. No later

<sup>1</sup> HAECKER, T. L., Investigations in milk-production, *Minn. Agr. Expt. Sta. Bull.* 140, 1914.

work has been so extensive as that of Haecker, but most of it has suggested somewhat lower values. The latest standard published by Morrison, which is based on a critical review of all of the experiments which have been made, states the requirement per pound of milk of a given fat percentage as a range, the upper figures of which are somewhat lower than those proposed by Haecker.

Overman and Gaines<sup>1</sup> have pointed out that the necessity of tables giving the energy requirement per pound of milk of various fat contents can be eliminated by first translating the yield into standard 4 per cent milk by their formula and then computing the requirement in terms of the energy value of this milk. This simplified procedure has much to commend it. For use in practice, it would require the adoption of a figure for the amount of total digestible nutrients or other measure of total nutritive energy which should be allowed per 100 Cal. of milk. Overman and Gaines suggest that the total nutritive value of the feeds be expressed in digestible energy and that 200 Cal. of this energy be allowed for each 100 Cal. in the milk.

In practice it is frequently found impossible to get the milking cow to consume as much total food during the peak of her lactation as is specified by her energy requirement. An inadequate intake under these conditions does not seem to limit production because the impulse to secrete causes the body reserves to be called upon. This is another reason for liberal feeding during the last part of gestation. The aim throughout lactation should be to prevent any marked loss of body weight, but fattening should be avoided except for the purpose of restoring any large loss which has occurred early in lactation, because milk secretion tends to decline if marked fattening occurs.

**316. The Importance of Food Fat in Lactation.**—While many experiments have shown that milk fat can be made from carbohydrates, the recent studies of Maynard and associates<sup>2</sup> suggest that a certain minimum level of fat is needed in the ration of the cow for the best milk yield. The studies showed that the removal

<sup>1</sup> OVERMAN and GAINES, *loc. cit.*

<sup>2</sup> MAYNARD and McCAY, *loc. cit.* MAYNARD, L. A., C. M. McCAY, H. H. WILLIAMS, and L. L. MADSEN, II. Further studies of the influence of different levels of fat intake upon milk secretion, *Cornell Agr. Expt. Sta. Bull.* 593, 1934.

of most of the fat from a dairy ration and its replacement by an isodynamic amount of starch cause a lowering of the yield, with no certain effect on the fat percentage. Some typical results are presented in Fig. 34. The normal fat ration consisted of hay, silage, and a grain mixture containing 5.8 per cent of fat, while the low fat ration was the same with the exception that, as a result of extraction, the fat content of the grain mixture was reduced to

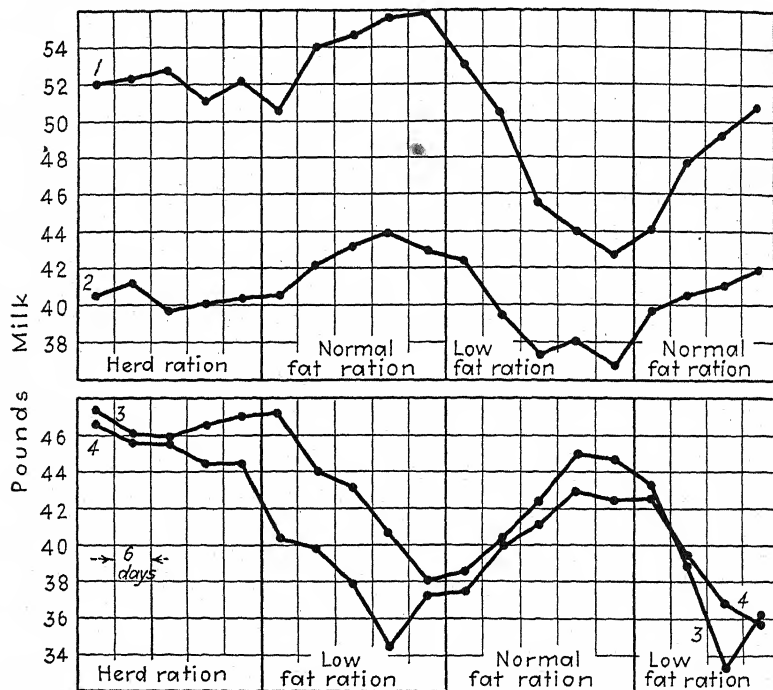


FIG. 34.—The influence of a ration low in fat on milk yield. (Maynard and McCay.)

0.66 per cent and an equivalent amount of starch added. The effects on production from the alternate feeding of these rations are clear.

In other experiments, no certain lowering occurred where the fat content of the ration was not reduced below the amount secreted in the milk. It seems probable that more physiological processes are involved where the food source is carbohydrate than where fat itself is available. Possibly, therefore, when the fat intake is markedly below the amount required to supply the

secreted product, the organism, which is working at full capacity, is unable to manufacture as much product when it is called upon to perform the additional work involved in the transformation of carbohydrate into fat. At any rate, it is interesting to note that the limitation expresses itself in the total yield of milk rather than affecting the percentage of its fat.

The statement is frequently made that an excessive amount of fat in the dairy ration tends to lower milk yield and some evidence for this viewpoint has been obtained by feeding certain oils. Undoubtedly the herbivorous animal has a lower tolerance for fat than Omnivora, but repeated experiments in our laboratory have failed to find any unfavorable effect from increasing the fat intake of the cow by natural feeds to levels considerably in excess of the amounts secreted in the milk, involving intakes close to a pound per day per 1000 lb. live weight.

**317. Energy Requirements for Other Species.**—In the absence of any measures of the amount of milk being secreted, no attempt is made to specify energy requirements for animals nursing young, separate from their total needs covering maintenance as well. On the basis of feeding trials, standards have been proposed by Morrison to cover the over-all requirements of sows, ewes, mares, and beef cows during lactation. In practice their total feed allowances are regulated in accordance with their apparent needs by giving sufficient amounts to keep them in good flesh during the nursing period.

#### THE PROTEIN REQUIREMENT

The minimum protein requirement for milk production is the amount secreted in the milk plus any catabolized as a specific accompaniment or result of the secretory process. In translating this minimum value into an actual requirement, account must be taken of the wastage in digestion and metabolism. It is customary to state the requirement for dairy cows in terms of digestible protein, since digestion coefficients are available for all of the common feeds for use in computing a ration which will meet a given requirement on this basis. The wastage in metabolism, however, is much less readily accounted for, since this is dependent upon the quality of the protein in terms of its amino-acid make-up.

**318. The Biological Value of Protein for Lactation.**—The differences in the biological values of various proteins and combinations

of them for growth, which modify the intakes required for this function according to the sources used, have been discussed (Secs. 254-259). That similar differences exist as regards milk secretion is to be expected, unless the mammary gland has special powers for the synthesis of amino acids not possessed by other tissues of the body. The actual determination of biological values for milk production, however, by the nitrogen-balance method involves experimental variables which frequently cannot be satisfactorily controlled or evaluated. Thus very few useful data have been obtained.

In discussing the determination of biological value for growth, the necessity was pointed out of using a level of intake below that capable of causing a maximum rate of growth in order to be sure that there was no excess intake which was being catabolized because unneeded, thus giving a biological value lower than the true one. Applied to the procedure for lactation, this means that the cow must be in negative nitrogen balance. Since the mature animal has only a limited ability to store protein, a state of equilibrium may mean that excess over that needed for her maximum secretory activity is being fed and therefore excreted. If a positive balance is shown, there is no way of knowing whether this represents all of the excess fed, and, if the excessive intake is large, some of it is undoubtedly excreted because of the limited capacity for storage. Thus biological values obtained with an animal in nitrogen equilibrium or in positive balance are likely to be lower than the true ones, and there is no way of knowing the extent to which this is the case. On the other hand, a large negative balance means that body tissue is being extensively catabolized and thus that the value is being determined upon the feed protein supplemented by body protein instead of upon the feed alone. The quantity of body protein utilized can be taken into account, but its qualitative effect in supplementing the feed protein cannot be evaluated.

These various facts suggest that the biological value should be measured with an animal in only slight negative balance. Such is the case, but there is no way of accomplishing it except by chance, for the balance is a function of the amount of milk secreted, which cannot be accurately estimated in advance, as well as a function of the unknown factor under study, *viz.*, the efficiency of the protein fed. The impossibility of knowing exactly how

much of the intake to allow for maintenance also enters. Thus the interpretation of data for biological values for milk secretion obtained by the nitrogen-balance method is always subject to uncertainty, and even the best planned experiments may result in data of little value. If the uncontrollable factors all operate in the same direction, the data are likely to be meaningless, but, if they tend to offset each other, a clear picture may be obtained.

The biological value of protein for milk production can be computed by the following formula:

$$\frac{\text{Nitrogen in milk} \pm \text{balance}}{\text{Digested nitrogen}} \times 100$$

This calculation gives the percentage of digested nitrogen which is utilized for milk production and accounts for any storage or loss by the body. The plus or minus sign in the numerator indicates that the balance is added if positive but subtracted if negative. Clearly any storage represents utilized nitrogen, while any loss should be subtracted to obtain the net amount utilized.

**319. Studies of Biological Value.**—Some 20 years ago Hart and Humphrey<sup>1</sup> carried out a series of nitrogen-balance studies with milking cows to ascertain the relative protein efficiency of various concentrates as supplements to different types of roughage. Milk protein proved more efficient as a supplement to corn stover than did corn or wheat protein. With corn stover and silage, gluten feed was inferior to milk, linseed-oil meal, casein, or distillers' grains. Using clover or alfalfa as the roughage, no differences were noted when various grains and vegetable-protein concentrates were compared. In most cases positive balances were obtained where the legume roughages were used and thus possible differences in protein efficiency may have been masked by too high levels of intake in relation to the production. The investigators recognized this possibility. They also felt, however, that the results could well be different with different types

<sup>1</sup> HART, E. B., and G. C. HUMPHREY, The relation of the quality of proteins to milk production, *J. Biol. Chem.*, **21**, 239-253, 1915; Further studies of the relation of the quality of proteins to milk production, *ibid.*, **26**, 457-471, 1916; The relation of the quality of proteins to milk production. III, *ibid.*, **31**, 445-460, 1917; Can home grown rations supply proteins of adequate quality and quantity for high milk production? *ibid.*, **38**, 515-527, 1919; Can home grown rations supply proteins of adequate quality and quantity for high milk production? II, *ibid.*, **44**, 189-201, 1920.

of roughages and thus that certain concentrates, such as gluten feed, might have an inferior value with corn stover, and yet not where legume hay was used because of the different mixtures thus provided.

To test the suggestion that legume hay might be superior to grass hay as a basis for rations for milking cows, Maynard and associates<sup>1</sup> conducted a series of nitrogen-balance experiments in which a ration of timothy hay, silage, and a concentrate mixture was compared with a ration of clover hay, silage, and concentrates. For illustrative purposes, the balance data from the experiments are given in a condensed form in Table XXXII. The same ingredients were used in the concentrate mixture in both cases, but the proportions were varied so that the higher protein of the clover would be balanced in such a way as to provide the same protein intake per unit of production from both rations. This, of course, involved a mixture of higher protein content with the timothy ration. Of the total nitrogen intakes given in the table, approximately one-half was furnished by the concentrates in the clover ration and approximately two-thirds in the case of the timothy combination. In each experiment the three cows received both rations in alternate periods. With the object of avoiding an excessive intake of protein which would result in a positive balance, the plan called for a total allowance which would provide a maintenance allowance of 0.7 lb. per 1000 lb. live weight, plus 120 per cent of the amounts which it was estimated would be secreted in the milk produced. It is noted that, contrary to what was hoped for, three of the balances were positive in the first experiment. The relatively large balance for cow 3 in the second trial was due to her failure to produce nearly as much milk as was estimated, illustrating an upsetting error which must be expected to occur occasionally in such an experiment. In the second experiment, all balances were positive, though small. Thus in the third experiment the level of protein intake was dropped to 0.6 lb. for maintenance and 110 per cent of the amount secreted. Fortunately, all the balances were negative and by small amounts. It is believed that this experiment came out better in this respect than could normally be expected.

<sup>1</sup> MAYNARD, L. A., R. C. MILLER, and W. E. KRAUSS, Studies of protein metabolism, mineral metabolism, and digestibility, with clover and timothy rations, *Cornell Agr. Expt. Sta. Mem.* 113, 1928.



The biological values were computed by the formula previously cited. The striking feature of these data is the similarity throughout. The majority of the figures lie between 55 and 65 per cent. If all of the data are considered, the differences between

TABLE XXXII.—THE BIOLOGICAL VALUE OF THE PROTEIN OF CLOVER AND TIMOTHY RATIONS FOR MILK PRODUCTION

Expt. no.	Trial no.	Cow no.	Daily amounts of nitrogen, g.					Biologi- cal value, %
			In- take in feed	Nitrogen outgo				
				Milk	Urine and feces	Total	Bal- ance	
1	1 (Clover)	1	326.26	113.23	215.00	328.23	- 1.97	64.1
		2	287.70	85.54	203.46	289.00	- 1.30	54.5
		3	363.77	102.88	242.34	345.22	+18.55	57.6
	2 (Timothy)	1	315.88	100.91	198.96	299.87	+16.01	62.8
		2	238.34	75.06	163.32	238.38	- 0.04	50.0
		3	367.71	100.77	224.30	325.07	+42.64	59.7
2	3 (Timothy)	4	262.47	101.48	156.56	258.04	+ 4.43	63.8
		5	235.45	82.61	137.63	220.24	+15.21	65.7
		6	224.02	70.54	140.31	210.85	+13.17	59.1
	4 (Clover)	4	268.98	95.58	164.35	259.93	+ 9.05	66.5
		5	241.86	80.34	155.25	235.59	+ 6.27	63.2
		6	220.83	64.12	152.86	216.98	+ 3.85	54.8
3	5 (Clover)	7	246.70	69.33	181.54	250.87	- 4.17	50.3
		8	245.08	63.94	181.83	245.77	- 0.69	49.6
		9	235.92	71.73	167.98	239.71	- 3.79	55.6
	6 (Timothy)	7	195.07	63.70	131.92	195.62	- 0.55	61.0
		8	191.86	68.53	131.01	199.54	- 7.68	58.3
		9	198.74	68.58	131.34	199.92	- 1.18	62.9

the averages are somewhat in favor of the timothy ration. If the positive balances are excluded, the same is true, and, if only the one comparison (Experiment 3) in which the balances were all negative is considered, the difference is larger. It cannot be concluded that any of these differences have a real significance other than to indicate rather clearly the lack of support for the

view that a ration based on legume hay is superior to one containing timothy instead, provided the proper adjustment is made in the concentrate mixture for the lower protein content of the grass hay.

This question of the quality of protein for milk production has recently been attacked by Morris and Wright<sup>1</sup> in nitrogen-balance experiments, in which data on milk yield, level of urinary nitrogen excretion, and amino-acid content of the feeds have been included as measures. They report differences in biological value for various feeds as supplements to a basal ration of oats, beet pulp, and straw. They use a formula for computing biological value which attempts to separate the nitrogen used for maintenance from that used for milk. Positive nitrogen balances are disregarded as being properly attributable to maintenance. In their last paper, assuming an arbitrary biological value of 75 for low-temperature dried-blood meal as a basis, they classify the comparative values of the various feeds studied as follows:

Feed	Biological Value
Fresh and dried spring grass	} ... 75 to 80 per cent
Grass silage (made from summer grass)	
Low-temperature dried-blood meal	
Fresh and dried autumn grass	} ..... 60 to 65 per cent
Bean and pea meals	
High-temperature dried-blood meal	} ..... 55 to 60 per cent
Meat meal	
Decorticated earth-nut cake	} 50 to 55 per cent
Decorticated earth-nut cake plus flaked maize	
Linseed cake	} ..... 45 to 50 per cent
Linseed-oil meal	

<sup>1</sup> MORRIS, SAMUEL, and NORMAN C. WRIGHT, The nutritive value of proteins for milk production. I. A comparison of the proteins of beans, linseed, and meat meal, *J. Dairy Research*, **4**, 177-196, 1933; II. A comparison of the proteins of blood meal, pea meal, decorticated earth-nut cake, and a mixture of decorticated earth-nut cake and flaked maize, *ibid.*, **5**, 1-14, 1933. MORRIS, SAMUEL, NORMAN C. WRIGHT, and ALEXANDER B. FOWLER, The nutritive value of proteins for milk production. IV. A comparison of the proteins of (a) spring and autumn grass, (b) grass conserved as silage (A.I.V. acid treated, molasses treated and ordinary untreated), and (c) grass conserved by drying, with notes on (i) the effect of heat treatment on the nutritive value and (ii) the supplementary relations of food proteins, *ibid.*, **7**, 97-121, 1936.

These values refer, of course, to combinations with the basal ration and not to the individual feeds.

It is believed that these various studies of protein quality which have been cited serve to emphasize the uncertainties and difficulties of the measures used, rather than providing much information which bears on the nutritive requirements in lactation or which can serve as a guide for the selection of efficient protein combinations in practice. Even if one assumes that all of the values reported are accurate for the rations fed, the usefulness of the data is limited by the fact that they provide little basis for estimating the efficiency of the many different combinations of feeds which are commonly used in practice. The fact that the protein efficiency changes with level of intake intensifies the problem in lactation, because the intake must be varied in accordance with a wide range of production. No information is directly obtainable as to the efficiency for milk secretion separate from maintenance. Previous discussion (Sec. 257) has indicated that in practice lesser differences in biological value are probable for the rations of Herbivora than for those of other species. This would appear to be especially true for the rations which are recognized as satisfactory in other respects for milk production. It is believed that a biological value of 60 per cent can generally be expected in these rations. Undoubtedly some have higher efficiencies, but there is no information upon which their exact values can be estimated, and present methods give little promise of obtaining such information.

### **320. Protein Requirements as Measured by Feeding Trials.—**

The protein allowances for milking cows recommended by current standards are based primarily on the results of feeding trials. Using combinations of feeds which are recognized to be satisfactory for milk production in other respects, the object has been to determine the protein intake which would certainly prove adequate for maximum production. Long-time feeding trials are essential for this purpose because an animal can keep up its production for an extended period, particularly in the first half of lactation, at the expense of its own tissues.

Haecker, whose long-time, pioneer studies have been referred to (Sec. 315), concluded that, allowing 0.7 lb. of digestible protein per 1000 lb. live weight for maintenance, an additional intake representing 138 per cent of that secreted in the milk was ade-

quate for satisfactory production and condition. He rejected a lower figure, because he felt that it did not keep the animals in the best condition. In setting up a standard for practice, Haecker increased the figure of 138 per cent to 175 per cent to allow a "factor of safety."

Since the pioneer studies of Haecker, many other experiments have been conducted to determine the protein requirements for milk production. Harrison and Savage<sup>1</sup> made extensive studies in which concentrate mixtures containing 12, 16, 20, and 24 per cent of protein were fed with timothy-clover-mixed-hay and corn silage. In two studies extending over two complete lactation periods, the ration containing the 16 per cent protein grain mixture gave as satisfactory results as those containing more protein. It was concluded that, when 0.7 lb. per 1000 lb. live weight is allowed for maintenance, an additional intake corresponding to 128 per cent of that secreted in the milk, the level supplied by the 16 per cent combination, is adequate. In a later experiment by the alternation system, a ration containing a 12 per cent protein mixture proved definitely inadequate as compared to the higher levels. While no significant differences between the three higher levels were noticeable for any one year, considering the experiments as a whole, the investigators noted a distinct indication of a slightly larger production where the 20 per cent mixture was fed than was the case with the 16 per cent mixture. The ration containing the 20 per cent combination supplied 150 per cent of the protein in the milk, after deducting the allowance for maintenance.

**321. Nitrogen-balance Data as Measures of Adequate Protein Nutrition.**—The fact that milk production can take place at the expense of body tissue has caused various workers to study the nitrogen balance as a further measure of the adequacy of intakes which were giving satisfactory production. When this has been done, it has generally been found that satisfactory production and nitrogen equilibrium can be maintained, at least over short periods, on intakes considerably below those recommended on the

<sup>1</sup> HARRISON, E. S., and E. S. SAVAGE, The effect of different planes of protein intake upon milk production, *Cornell Agr. Expt. Sta. Bull.* 540, 1932. HARRISON, E. S., E. S. SAVAGE, and S. H. WORK, The effect of different planes of protein intake upon milk production. II. Further comparisons of 16-, 20-, and 24-per cent mixtures, *ibid.*, 578, 1933.

basis of feeding trials. An example of the use of the nitrogen-balance method in measuring protein requirement is presented by an experiment by Fries and associates.<sup>1</sup> Allowing 0.6 lb. of protein per 1000 lb. live weight for maintenance, various additional levels calculated as percentages of the milk yield were fed in different periods. It was found that the cows were approximately in nitrogen equilibrium when the nitrogen intake above the calculated maintenance was from 111 to 117 per cent of the output in the milk. There was evidence, however, that this intake tended to lower the level of secretion.

When the protein intake is reduced below the requirement, there may be a drop in production, or its level may be maintained at the expense of the body and be reflected in a negative balance. Undoubtedly both take place over any long period on an inadequate protein intake. Such was found to be the case in a supplementary study of the ration containing the 12 per cent protein grain mixture used by Harrison and Savage. Clearly nitrogen-balance data alone are not an adequate measure of a satisfactory protein intake for milk production, but they provide useful supplementary information in connection with feeding trials.

Several of the nitrogen-balance studies have furnished some pertinent information relative to the maintenance requirement for protein during lactation, as is discussed by Maynard and associates.<sup>2</sup> In many instances where positive nitrogen balances have been obtained, subtracting from the intake of digestible protein an allowance of 0.7 or even 0.6 lb. for maintenance has left a remainder which is less than the amount of protein found in the milk. Since a conversion efficiency greater than 100 per cent is impossible such a finding must mean that the amount actually used for maintenance was less than calculated. If the customarily used maintenance values are correct for the nonproducing animal, then they must be lower under certain conditions of production. Such findings from short-time balance studies are interesting, but it is unsafe to interpret them as having general application in practice.

**322. Protein Allowances for Milking Cows in Practice.**—The data of the many experiments dealing with the protein require-

<sup>1</sup> FRIES, J. AUGUST, WINFRED W. BRAMAN, and MAX KRISS, On the protein requirement of milk production, *J. Dairy Sci.*, 7, 11-23, 1924.

<sup>2</sup> MAYNARD, MILLER, and KRAUSS, *loc. cit.*

ments for milk production, a few of which have been cited, are by no means in agreement. Close agreement among experiments conducted under different conditions is hardly to be expected in view of the variables concerned in protein utilization. From a consideration of the many data, the writer believes that, basing the maintenance requirement on an intake of 0.6 lb. of digestible protein for a 1000-lb. cow, the minimum allowance for production should be 125 per cent of that secreted in the milk. It is believed that an intake of 150 per cent is distinctly preferable as a general recommendation and that an even higher level may give superior results under certain conditions. Despite the evidence that lower intakes have resulted in excellent production in several experiments, liberal allowances seem wise as general recommendations because of the indications that they may produce somewhat more milk under many conditions, because of the lack of certain knowledge as to the extent of the differences in biological value which may occur, and because of the possible effect of liberal intakes in improving energy utilization (Sec. 218). From the standpoint of practice, however, the possible advantage of the higher level of intake may be overbalanced in many situations by the extra cost of high-protein feeds.

The protein content of milks of different fat percentage may be calculated by the relations set forth by Overman and Gaines (Sec. 313) or one may consider the protein percentage increases by 0.42 for each 1 per cent increase in fat, as found in studies at the Ohio Experiment Station.

**323. Protein Requirements for Other Species.**—There is no basis for estimating the requirements of animals nursing young in terms of their actual lactation performance. Rations having a nutritive ratio of approximately 1 : 7 should contain an adequate proportion of protein, with the exception that a somewhat narrower ratio may be preferable for brood sows. It is noted in Table XXIX that the protein-Calorie ratios for the milk of the different species of farm animals are not markedly different.

#### THE MINERAL REQUIREMENTS

Aside from the elements supplied by common salt, the only minerals which require consideration in rations for milk secretion are calcium and phosphorus. The others which occur in milk are always supplied adequately by the commonly used feeds.

**324. Calcium and Phosphorus.**—Compounds of calcium and phosphorus make up approximately 50 per cent of the ash of milk and thus its secretion requires a liberal supply of them in the ration. A cow producing 10,000 lb. of milk during her lactation secretes in it approximately 12 lb. of calcium and 10 lb. of phosphorus, and, at the peak of her production, the daily calcium output may exceed 30 g., with a somewhat smaller figure for phosphorus. These figures, however, do not represent the total requirements of the lactating animal because of the needs for maintenance, as well as for the pregnancy which normally occurs in the course of the lactation, and because of the fact that there is a large wastage of calcium and phosphorus in metabolism.

**325. The Cycle of Calcium and Phosphorus Metabolism.**—Owing primarily to the pioneer and extensive work of Forbes and associates<sup>1</sup> begun in 1912 at the Ohio Experiment Station, it has come to be recognized that the natural and significant unit of time in the calcium and phosphorus metabolism of the dairy cow is the annual cycle of lactation and gestation. By means of balance experiments, Forbes found that the most liberal feeding of calcium and phosphorus would commonly not meet the current needs of liberally producing cows during the first part of the lactation but that, toward the end of the lactation and particularly during the dry period, the earlier losses from the body ceased and were replaced by a storage of the elements. A similar cycle has been found to occur in lactating women, and there is evidence that the same is true for the rat and dog. Probably this depletion and restoration of the bone reserves is a common occurrence in all mammals during the lactation cycle.

By continuous balance studies over the entire cycle of lactation and gestation both Ellenberger and coworkers<sup>2</sup> and Forbes and

<sup>1</sup> FORBES, E. B., and F. M. BEEGLE, The mineral metabolism of the milch cow. I, *Ohio Agr. Expt. Sta. Bull.* 295, 1916. FORBES, E. B., F. M. BEEGLE, C. M. FRITZ, L. E. MORGAN, and S. N. RHUE, The mineral metabolism of the milch cow. II, *ibid.*, 308, 1917. FORBES, E. B., J. O. HALVERSON, and L. E. MORGAN, The mineral metabolism of the milch cow. III, *ibid.* 330, 1918.

<sup>2</sup> ELLENBERGER, H. B., J. A. NEWLANDER, and C. H. JONES, Calcium and phosphorus requirements of dairy cows. I. Weekly balances through lactation and gestation periods, *Vt. Agr. Expt. Sta. Bull.* 331, 1931; Calcium and phosphorus requirements of dairy cows. II. Weekly balances through lactation and gestation periods, *ibid.*, 342, 1932.

coworkers<sup>1</sup> have shown that liberally producing cows may be in negative calcium and phosphorus balance for extended periods early in lactation and still end the cycle with a surplus of the minerals, as a result of storage later in the lactation and during the dry period. Data from one of the Vermont studies are presented in Fig. 35. This chart gives the record of a mature Ayrshire cow from the beginning of lactation over a period of

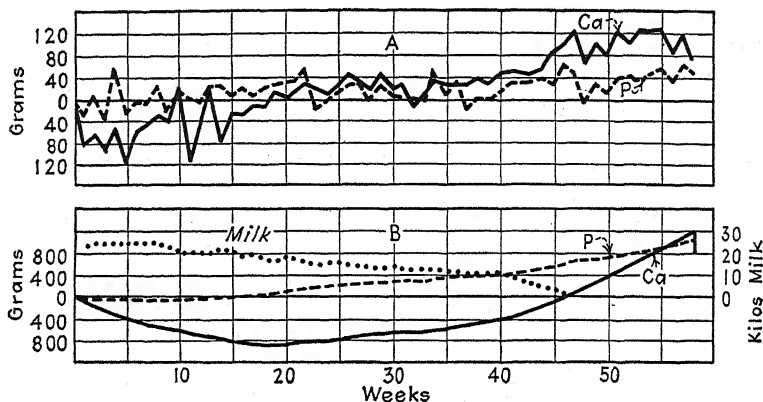


FIG. 35.—Calcium and phosphorus balances throughout the lactation cycle. A. Weekly balances of calcium and phosphorus. B. Milk yield and cumulative balances of calcium and phosphorus. (Ellenberger, Newlander, and Jones.)

58 weeks until calving. She was in milk for 47 weeks and produced 11,254 lb. during this period. Her ration consisted of timothy hay, corn silage, and grain during the winter, but during the summer the silage was discontinued and fresh-cut grass was largely substituted for the hay. The average daily intakes of calcium and phosphorus were approximately 45 and 60 g., respectively.

The weekly balances presented in the upper part of the chart, while showing considerable fluctuations, reveal losses of the elements, particularly of calcium, during the early weeks, in contrast to storages which regularly occurred during the last half of the period. The results over the period as a whole are better shown in the lower part of the chart where the cumulative balances are presented. It is noted that at 20 weeks the cow had lost over 800 g. of calcium and that she did not regain her losses completely

<sup>1</sup> FORBES, BLACK, BRAMAN, FREAR, KAHLENBERG, McCLURE, SWIFT, and VORIS, *loc cit.*



until the forty-sixth week, a point which happened to coincide with the close of the lactation. During the following weeks, the calcium store accumulated rapidly. The curve for phosphorus shows a net loss through 12 weeks, followed by a storage which was accelerated as the decline in milk flow became more rapid. The dips in both curves at the end represent a subtraction of the minerals in the calf and placenta, a deduction which left the cow with net gains of 795 g. of calcium and 813 g. of phosphorus. Clearly, negative balances early in lactation do not necessarily mean that the ration is inadequate in calcium and phosphorus for the cycle as a whole, and it would appear that the utilization of reserves early in lactation is a normal process, not harmful to the animal provided the losses are not too great and provided they are fully made good later.

**326. Effects of Calcium and Phosphorus Deficiencies.**—If, due to an inadequate ration, the demands for calcium and phosphorus during lactation are in excess of the reserve supply, or, if the losses are not made good, both the animal and her production eventually suffer. With rations which are extremely low in either of the minerals, the bones may become so impoverished in them as to break, destroying the usefulness of the animal. In less severe situations, the bones may become progressively weakened in succeeding lactations due to incomplete restoration of the losses, and thus it becomes increasingly difficult for the animal to keep up her milk flow. The production may fall off more rapidly than normal in a given lactation, or it may fail to reach previous levels in succeeding lactations. The effect of small deficiencies of calcium and phosphorus may not become evident until after two or three or more years, the essential effect being to shorten the productive life of the animal.

Striking evidence of the effect on production of inadequate calcium and phosphorus nutrition has come from studies in the phosphorus-deficient areas. In South Africa<sup>1</sup> the feeding of bone meal to cows on deficient pasture increased the milk production by 40 per cent, while in Minnesota<sup>2</sup> the addition of phosphorus increased the yield by 50 to 146 per cent. It is estimated by the

<sup>1</sup> THEILER, ARNOLD, H. H. GREEN and P. J. DU TOIT, Phosphorus in the live stock industry, *J. Dept. Agr., Union S. Africa*, 8, 460-504, 1924.

<sup>2</sup> ECKLES, C. H., T. W. GULLICKSON, and L. S. PALMER, Phosphorus deficiency in the rations of cattle, *Minn. Agr. Expt. Sta. Tech. Bull.* 91, 1932.

Minnesota authorities that the production losses in phosphorus-deficient areas cost the farmers over a million dollars in the five years prior to the discovery of the cause of the trouble. Similar effects from severe calcium deficiency have been reported from Florida by Becker and coworkers.<sup>1</sup> Owing to the very low content of this element in the roughages, broken hips and ribs were not an uncommon occurrence in the lactating animals. When the calcium intake was raised by the addition of bone meal the yield, per lactation, increased by 50 per cent, and the cows became more persistent producers. When they were slaughtered at the close of the experiment, tests of the bones revealed an excellent state of mineral storage. Depleted bones from an animal on the calcium-deficient ration are shown in Fig. 36.

To what extent a less severe and thus unnoticed bone depletion may limit milk production and productive life, by reason of rations inadequate in calcium and phosphorus, is unknown. In fact, it is possible that, even with the best mineral nutrition we know how to provide, productive life may be shortened by failure to meet the physiological demands of extremely high production. Evidence on the question would be very difficult to obtain.

The extent of the losses from the bones which can occur early in lactation without immediate detriment to production or the bones themselves, provided the losses are made good later, depends upon the state of the reserves at the start. It is clear that, in considering the entire cycle as a unit, we cannot ignore its various parts. The early losses of calcium and phosphorus can be kept at a minimum by liberal intakes at this time; in fact, some investigators have reported that cows producing from 60 to 80 lb. a day have been held in equilibrium either by the use of natural feeds rich in the minerals or by the addition of mineral supplements. While the majority of the experimental results show that such success is unusual, it is desirable to feed the minerals liberally so that excessive losses will certainly be avoided. Vitamin D is not effective in preventing these losses (Sec. 332).

**327. Calcium and Phosphorus Requirements.**—As a basis for estimating the requirements of the dairy cow, there are the annual balances previously discussed, data from slaughter experiments as

<sup>1</sup> BECKER, R. B., W. N. NEAL, and A. L. SHEALY, Effect of calcium-deficient roughages on the milk yield and bone strength of cattle, *J. Dairy Sci.*, 17, 1-10, 1934.

to the state of the bones after successive lactations and the results of long-time feeding trials. From the Vermont data, it is clear that 45 g. of calcium and 60 g. of phosphorus per day were certainly adequate for a cow producing around 11,000 lb. But they

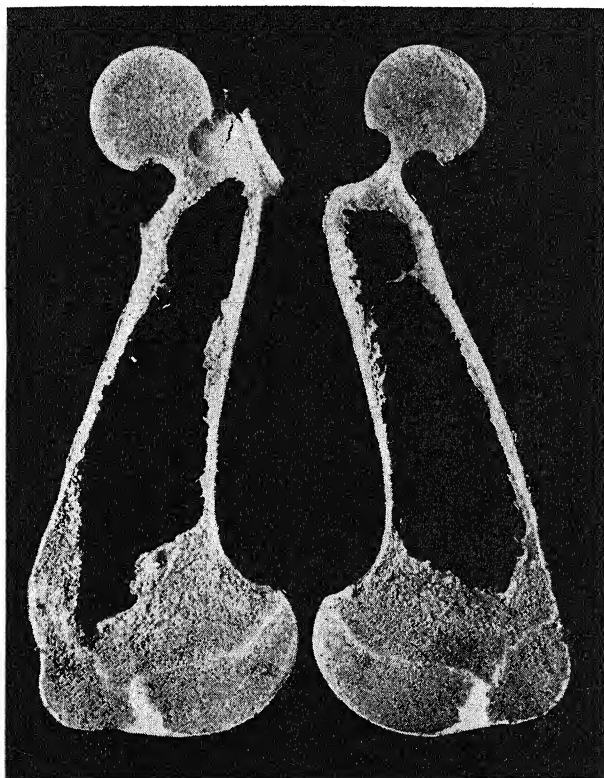


FIG. 36.—Humeri depleted by a ration low in calcium. These bones were taken from a first-calf heifer. (Courtesy of R. B. Becker, Fla. Agr. Expt. Sta.)

cannot be considered minimum values, especially in the case of phosphorus, in view of the net storages obtained. For a similar production, Forbes and associates, from their annual balances, express the view that a ration containing 39 g. of calcium and 33 g. of phosphorus is adequate. The phosphorus figure rests on the more certain basis. From slaughter experiments, Meigs and coworkers<sup>1</sup> have reported that 25 g. of calcium was not quite

<sup>1</sup> MEIGS, EDWARD B., WILLIAM A. TURNER, EDWARD A. KANE, and LEO A. SHINN, The effects on calcium and phosphorus metabolism in dairy

adequate to maintain bones intact in successive lactations in cows producing around 6600 lb. of milk annually. In contrast Hart and coworkers<sup>1</sup> report that the bones of cows producing 10,000 to 12,000 lb. annually remained intact up to the fourth lactation on an average daily calcium intake of 25 to 28 g. and a phosphorus intake of 28 g. In this experiment timothy hay ranging from 0.35 to 0.5 per cent calcium was used. The lower value was employed in calculating the calcium figures reported. These calcium and phosphorus intakes could be supplied by rations which contain approximately 0.25 per cent of each of the elements in the dry matter. The figures suggested by Forbes and coworkers would require around 0.32 per cent of calcium and 0.3 per cent of phosphorus.

It is evident that expressing the requirements as averages for the cycle as a whole does not take account of the greatly varying demands according to the stage of lactation. While it is generally impossible to prevent losses from the bones during the height of secretion, it seems reasonable to provide intakes at this time in accordance with the amounts being secreted. Milk contains approximately 0.5 g. of calcium and 0.4 g. of phosphorus per pound. The intakes specified must allow for the wastage in assimilation, and the total allowances must also cover the needs of maintenance. The data for arriving at these figures are meager. Using 10 g. of phosphorus as the maintenance requirement for a 1000-lb. cow, Huffman and associates<sup>2</sup> concluded that there should be an additional allowance of 0.75 g. per pound of milk. The latter figure provides nearly double the amount secreted in the milk and this provision seems wise in view of the losses in digestion and metabolism. On the same basis the calcium allowance per pound of milk should be 0.95 g., in addition to an estimated maintenance requirement of 8 g.

On the above basis a 1000-lb. cow producing 60 lb. of milk at the height of her lactation would require 65 g. of calcium and 55 g.

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cows, of feeding low-calcium rations for long periods, *J. Agr. Research*, **51**, 1-26, 1935.

<sup>1</sup> HART, E. B., F. B. HADLEY, and G. C. HUMPHREY, The relation of nutrition to contagious cattle abortion, *Wisc. Agr. Expt. Sta. Research Bull.* 112, 1932.

<sup>2</sup> HUFFMAN, C. F., C. W. DUNCAN, C. S. ROBINSON, and L. W. LAMB, Phosphorus requirement of dairy cattle, when alfalfa furnishes the principal source of protein, *Mich. Agr. Expt. Sta. Tech. Bull.* 134, 1933.

of phosphorus. Huffman and associates concluded that the phosphorus intake should not go below 17 g. even when the cow is dry because of the needs for gestation as well as for maintenance. The same should be true for calcium. Feeding in accordance with the allowances previously mentioned for maintenance and milk, with the 17 g. as the lower limit for either mineral, would provide, in the case of the cow producing 10,000 lb., average intakes for the complete cycle close to those found adequate by Forbes and associates. This system of feeding would seem to be a satisfactory way of meeting the calcium and phosphorus requirements of the dairy cow.

There is no adequate basis for estimating the calcium and phosphorus needs of lactating ewes, sows, mares, or beef cows. It is believed that rations containing the percentages of the minerals that have been suggested for the period of rapid growth (Sec. 270) in these species should be adequate for lactation also. Particular attention is needed in the case of the ewe and sow since their milk is considerably richer in both calcium and phosphorus than is that of other species (Table XXIX). In providing for the needs of lactating animals in practice, account must be taken of the composition of the feeds available (Sec. 111), and, in making up the ration, the same principles mentioned in connection with growth (Sec. 272) should apply. A detailed discussion of these practical aspects is to be found in a bulletin by the writer.<sup>1</sup>

**328. Salt.**—As is evident in Table XXX milk contains considerable amounts of both sodium and chlorine. Several studies have shown that milk secretion is decreased by depriving cows of salt, but the specific amount needed cannot be stated (Sec. 120). In the previously mentioned continuous balance experiments of Forbes and coworkers (Sec. 325) salt was included as 1.5 per cent of the concentrate mixture fed the lactating cows. The intakes, which ranged from 50 to 88 g. daily for different animals, were certainly adequate as shown by the annual balances and probably unnecessarily high. One to two ounces of salt daily should be adequate for milking cows. The addition of 1 per cent of salt to the grain mixture serves the double purpose of providing this needed mineral and increasing the palatability of the mixture.

<sup>1</sup> MAYNARD, L. A., Meeting the mineral needs of farm animals, *Cornell Agr. Expt. Sta. Ext. Bull.* 350, 1936.

It is a good plan to give the cows free access to salt in addition. By this means those animals which crave more than is provided in the grain mixture can get it and this procedure should certainly be followed when little grain is being fed.

There are no accurate data on the salt requirements of other lactating animals. The common practice of feeding salt *ad libitum* undoubtedly insures an adequate intake.

#### THE ROLES OF VITAMINS IN LACTATION

Vitamins are important in lactation both as essential nutrients for the physiological process involved and as components of the secretion itself.

**329. Vitamin A Value of Milk.**—The cow on its natural herbivorous ration receives vitamin A only in the form of carotene. A portion of the carotene ingested is secreted in milk as such and a portion is transformed into vitamin A and so secreted. The more yellow the milk and butter the larger the amount of carotene present, but this is not a true measure of vitamin A value for it gives no information as to the amount of the vitamin present as such. Jersey and Guernsey milk has much more color than Holstein milk because these cows convert a smaller proportion of their carotene intake into the vitamin. Most of the vitamin value of their milk is due to carotene, whereas Holstein milk contains less of the pigment and more of the colorless vitamin. As a result, when the cows are fed the same ration Holstein butter fat has fully as high a vitamin A value as that from Jerseys or Guernseys despite the marked difference in color, as has been shown by Hathaway and Davis<sup>1</sup> and by Baumann and associates.<sup>2</sup> The difference in degree of carotene conversion is also reflected in larger amounts of pigment in the adipose tissue and skin secretions. The extent of the conversion varies among individuals as well as among breeds. Those species which secrete a colorless milk (Sec. 310) make a complete conversion and thus the vitamin value of their fat may be very high though no color is present.

<sup>1</sup> HATHAWAY, I. L. and H. P. DAVIS, The vitamin A content of skim milk, standardized milk, and cream from different breeds of cows, *Nebr. Agr. Expt. Sta. Research Bull.* 69, 1933.

<sup>2</sup> BAUMANN, C. A., H. STEENBOCK, W. M. BEESON, and I. W. RUPEL. Fat-soluble vitamins. XXXIX. The influence of breed and diet of cows on the carotene and vitamin A contents of butter, *J. Biol. Chem.*, **105**, 167-176, 1934.

**330. Relation of Feed to Vitamin A Value of Milk.**—Aside from the contribution which can temporarily be made by the reserves, the vitamin A value of milk is entirely dependent upon the amount present in the feed and very large variations in the potency may accordingly occur. The amount found in the milk may be several times as great on feeds high in carotene as on feeds which contain very little. Of the natural feeds, pasture results in the richest milk but nearly as large a potency can be obtained by feeding properly cured alfalfa, dried grass, or corn silage. High potencies are also produced by feeding concentrates of the vitamin.

Despite the large differences in the vitamin A potency of milk according to the nature of the diet, the percentage of the intake that appears in the milk is very small. Russell and associates<sup>1</sup> have reported that daily intakes ranging from 900,000 to 1,200,000 U.S.P. X (1934) units, supplied by a ration of alfalfa hay, corn silage, and corn, resulted in a potency of approximately 2500 units per quart. In another test where the daily milk production was somewhat larger, a daily intake of 950,000 units produced 1600 units per quart in the milk. In no case, according to the investigators, did the output exceed 3.5 per cent of the intake. Recoveries up to 10 per cent have been reported where much smaller daily intakes than those used by Russell and associates were fed. It is evident from these and other studies that the percentage recovery decreases with increasing intake and that there is a maximum milk potency which cannot be exceeded by further intakes of the vitamin or its precursor. This maximum level is apparently reached on pasture.

**331. Vitamin A Requirements.**—It is clear that the animal nursing young should receive a liberal intake of vitamin A in order that its milk may contain an adequate amount for the nutrition of its offspring, at least until they are able to obtain this vitamin from supplementary foods. This is particularly true because the newborn has little reserve of the vitamin in its body even though the mother may have been fed liberally during pregnancy. The latter does, however, provide stores in the body of the mother which will be drawn upon for her milk and which lessen accordingly the necessity of large intakes during lactation. The

<sup>1</sup> RUSSELL, WALTER C., M. W. TAYLOR, D. F. CHICHESTER, and LOGAN T. WILSON, The relation between the vitamin A content of the dairy ration and of milk, *N. J. Agr. Expt. Sta. Bull.* 592, 1935.



disastrous results obtained by Converse and Meigs in young calves fed milk and hay low in the vitamin have been discussed (Sec. 275). Unless the cow is fed for an extended period on very poor roughage, there should be no practical problem in meeting the needs of the suckling offspring particularly when the calf is given access early to green, leafy roughage. The same should be true for lambs and colts. In the case of pigs, which are dependent solely on their mother's milk for a relatively longer period, the proper feeding of the mother may be of greater importance. Her needs should be met by selecting her ration in accordance with the same principles mentioned for growing pigs (Sec. 275).

The mature animal requires vitamin A for various body functions but whether this need increases for the specific function of lactation apart from the demand for secretion in the milk has not been definitely proved. Clearly, the first effect of a deficient diet is a lowering of the vitamin content of the milk. One would expect any eventual effect on production itself to be accompanied by signs of deficiency in the lactating animal. Rations which are adequate in vitamin A for reproduction should certainly support normal lactation, although they might not provide a milk rich in the factor.

**332. Vitamin D.**—The early studies of Forbes (Sec. 325), showing that the negative calcium and phosphorus balances of the height of lactation could not be eliminated by increasing the content of these minerals in the ration, were made before vitamin D was known. With the discovery of its role in the improvement of calcium and phosphorus retention, investigations were undertaken with the expectation that the addition of this vitamin to the ration would do away with the negative balances previously noted. Some initial experiments with goats supported this viewpoint, but more extensive studies with cows gave only negative results. Neither sun-cured hay, cod-liver oil, irradiated yeast, nor any other source of vitamin D, including direct irradiation, was found effective in preventing the losses of calcium and phosphorus which occurred in the liberally producing cow early in lactation. Extensive studies of this question have been made by Hart and associates.<sup>1</sup> Why the vitamin is markedly useful in

<sup>1</sup> HART, E. B., H. STEENBOCK, E. C. TEUT, and G. C. HUMPHREY, Dietary factors influencing calcium assimilation. XI. The influence of cod-liver



promoting calcium and phosphorus storage in growing calves, yet ineffective in preventing losses during lactation, cannot be explained at present. In the case of the irradiated yeast, at least, the ineffectiveness is not due to a failure of absorption, according to Light and associates.<sup>1</sup> Hart<sup>2</sup> considers the parathyroid to be the controlling influence. Whatever may be the explanation, the various experimental data fail to indicate that vitamin D has any marked value in promoting calcium and phosphorus metabolism in the lactating cow.

The question has not been critically studied in other farm animals. In the case of women, some investigators have reported a marked improvement in the calcium and phosphorus balances of lactation by feeding cod-liver oil, while others have noted little effect. Clearly, neither a uniform nor a complete response can be counted on.

No milk produced from natural feeds is a rich source of vitamin D in terms of the needs of the growing young. As a result of many studies, there is rather complete information as to the range of vitamin D content found in the milk of different breeds and under different conditions. For example, the following data were obtained by Bechtel and Hoppert<sup>3</sup> from a two-year study of the milk of the Guernseys and Jerseys in the Michigan College herd. In tests at monthly intervals, vitamin D values ranging from 4.8 to 43.8 U.S.P. units per quart were found in Guernsey milk while for Holsteins the range was from 3.1 to 28 units. The highest value occurred in the summer and the lowest in winter, and there was a close correlation between the hours of sunshine and the vitamin level, indicating that sunlight was the principal

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oil on calcium metabolism of milking cows, *J. Biol. Chem.*, **84**, 359-365, 1929; XII. A study of the influence of hays cured with varying exposure to sunlight on the calcium metabolism of milking cows, *ibid.*, **84**, 367-376, 1929. HART, E. B., H. STEENBOCK, O. L. KLINE, and G. C. HUMPHREY, Dietary factors influencing calcium assimilation. XIII. The influence of irradiated yeast on the calcium and phosphorus metabolism of milking cows, *ibid.*, **86**, 145-155, 1930.

<sup>1</sup> LIGHT, R. F., L. T. WILSON, and C. N. FREY, Vitamin D in the blood and milk of cows fed irradiated yeast, *J. Nutrition*, **8**, 105-111, 1934.

<sup>2</sup> HART, E. B., Nutrition and milk production, *J. Am. Med. Assoc.*, **99**, 152, 1932.

<sup>3</sup> BECHTEL, H. ERNEST and C. A. HOPPERT, A study of the seasonal variation of vitamin D in normal cow's milk, *J. Nutrition*, **11**, 537-549, 1936.

factor involved in the variations. There was little difference in the potencies of the butterfats of the two breeds, thus the Guernsey milk was richer because of its higher fat content.

These variations in potency indicate that the vitamin D content of milk is under physiological control, but no system of feeding, other than massive intakes of the vitamin, has been found effective in increasing the potency above the normal summer level. Kon and Henry<sup>1</sup> have reported that the addition to a winter ration of 2 lb. daily of cacao-shell meal containing 35 International units of the vitamin per gram increased the potency of Shorthorn butter two and one-half times, resulting in a product comparable to the summer milk. Cod-liver oil concentrates and irradiated ergosterol are more effective, but very large intakes are required to produce a relatively small change in the milk. Feeding cod-liver oil to the human mother will not cure rickets in the suckling infant. Experiments in which the cow has been irradiated have generally failed to increase the vitamin D content of the milk, but the recent studies of Campion and associates<sup>2</sup> have proven that the higher potency of milk produced by cows on pasture is due entirely to the action of sunshine.

The foregoing discussion indicates that little can be accomplished by giving consideration to the vitamin D intake of the lactating cow under the usual conditions of practice. No beneficial effect can be obtained on her calcium and phosphorus metabolism, and her milk will remain relatively low in vitamin content despite the best ration of natural feeds that can be selected. The feeding of concentrated sources of the vitamin is an inefficient and expensive way of increasing its content in the milk. From the standpoint of the nursing young, it is more practicable to insure their protection against rickets by appropriate supplementary feeds (Sec. 272). It is important, however, to bear in mind the marked difference which occurs between summer and winter milk of the cow, making winter the critical period. The extent to which these various considerations apply to other farm animals is unknown.

<sup>1</sup> KON, STANISLAW K., and KATHLEEN M. HENRY, The effect of feeding cacao shell to cows on the vitamin D content of butter (milk), *Biochem. J.*, **29**, 2051-2056, 1935.

<sup>2</sup> CAMPION, JOHN EDWARD, KATHLEEN MARY HENRY, STANISLAW KAZIMIERZ KON, and JAMES MACKINTOSH, The source of vitamin D in summer milk, *Biochem. J.*, **31**, 81-88, 1937.

**333. Vitamin D Milk.**—The best milk that can be produced in summer, or on any ration of natural feeds, falls far short of meeting the requirements of children for protection against rickets.

The recognition of this fact and the discovery of methods of enriching milk to the effective level by direct irradiation or by feeding irradiated yeast to the cow has resulted in the production and use of vitamin D milk. This development has received the approval of medical authorities and nutritionists because, despite the knowledge of other effective methods of preventing rickets, the disease persisted unduly. It was felt that if the vitamin were adequately supplied in a product which formed a considerable part of the daily diet of children, a more certain way of insuring the needed intake would be provided.

By the direct irradiation of milk, a potency of 135 or more U.S.P. units per quart can be imparted. By feeding a sufficient amount of irradiated yeast to the cow a milk containing 430 units per quart is obtained. Vitamin D milk is also produced by the direct addition of a cod-liver oil concentrate or ergosterol activated by cathode rays. The latter products now on the market are standardized to contain 400 units per quart. There have been many studies of the relative value of these different kinds of milk for the child, with rather divergent results. The primary question at issue has been as to whether the relative value of the milks is actually represented by their potencies expressed in rat units. The question of the multiple nature of the vitamin is involved (Sec. 152). It is impossible to present here the various experimental evidence which has been adduced. The reader is referred to the extensive review by Jeans<sup>1</sup> presented to the Committee on Foods of the American Medical Association and authorized for publication by the Committee.

Jeans states that, exclusive of purely laboratory products, there may be only two varieties of vitamin D; an animal source represented by such products as irradiated milk and cod-liver oil and a vegetable source such as irradiated yeast and irradiated ergosterol. It is concluded that a vitamin D milk which contains 135 units per quart from an animal source will prevent rickets but that this amount of the vitamin is close to the minimum effective

<sup>1</sup> JEANS, P. C., Vitamin-D milk: The relative value of different varieties of vitamin D milk for infants: A critical review, *J. Am. Med. Assoc.*, 106, 2066-2069, 2150-2159, 1936.

level. Such a level is not considered adequate for the best growth and calcification. Jeans further concludes that animal-source vitamin D in the amount provided by one standard teaspoonful of average high grade cod-liver oil or in milk containing 400 units per quart is adequate for growth and calcification but that the minimum amount which is adequate is unknown. Regarding vegetable-source vitamin D, it is stated that the products here included have not been employed in such a manner as to determine the minimum protective dose against rickets or the amount needed for growth and calcification.

Obviously the potency required in the milk depends upon the amount consumed daily. In considering what this potency should be the Committee of the American Medical Association refers to the daily "customary quantity" as  $1\frac{1}{2}$  oz. per pound of body weight for early infancy and  $1\frac{1}{2}$  pt. per child later.

The production of vitamin D milk is controlled by patents. Based on the usual costs of milk production, the increased outlay required for the special product is a substantial one demanding an increased return accordingly. In a review of the recent developments with respect to vitamin D milk, Krauss and Bethke<sup>1</sup> have described the details of the various methods of production and given information as to materials and equipment required, the costs, and other data of special interest to the producer and distributor.

**334. Vitamin B (B<sub>1</sub>).—**The need of vitamin B for successful lactation in the rat, as measured by the growth and survival of the young, has been shown by several investigators. This requirement during lactation is several times that during growth. It appears that a deficiency affects the amount of secretion and not merely its vitamin content, though it is difficult to determine this with certainty by the rat technique. The effect is independent of the influence of the vitamin on food intake. The cost and difficulty involved in obtaining suitable rations have prevented studies of the needs of lactating farm animals for vitamin B. In view of the nature of their feeds, it seems improbable that any deficiency could exist in the commonly fed rations.

The rat experiments have indicated that the amount of vitamin secreted in the milk is reduced when the mother's diet is deficient.

<sup>1</sup> KRAUSS, W. E., and R. M. BETHKE, New developments in the field of vitamin D milk, *Ohio Agr. Expt. Sta. Bimonthly Bull.* 184, 3-12, 1937.

It seems clear from the observations of Andrews<sup>1</sup> that the same is true for women. He reported that beriberi developed in pups when they were nursed by women who had previously lost their infants from this same disease. The women had received a diet consisting principally of polished rice. When other nursing infants showed signs of beriberi, improvement was brought about by an appropriate change in the diet of the mother. It appears from these observations that the mother's milk may become very deficient before the mother herself is affected.

Shortly after 1920 evidence was reported that, as measured by the growth of rats, the vitamin B content of the milk of the cow could be decreased by feeding a ration poor in the vitamin. This evidence may still stand despite the fact that the experiments were made before the multiple nature of the vitamin B complex was appreciated, and despite the evidence for the rumen synthesis of the vitamin (Sec. 161). But recent studies have certainly shown that there is at least an upper limit beyond which the vitamin B content of milk cannot be increased by feeding. Gunderson and Steenbock,<sup>2</sup> using a basal ration of hay and a concentrate mixture consisting mostly of corn, oats, and bran, were not able to increase the potency of the milk secreted by cows or goats when rich sources of vitamin B were added. They found no essential differences in the vitamin content of the milk of Holstein, Guernsey, or Durham cows, nor did the stage of lactation have any influence. These observations have been confirmed by other workers. Cow's milk as normally produced is rather low in vitamin B in terms of human needs. These experiments indicate the futility of attempts to increase its content via the cow's ration.

**335. Flavin Factor.**—The studies of Hussemann and Hetler<sup>3</sup> have shown that flavin as well as vitamin B is essential for lactation in the rat. It cannot be said that we have any specific knowledge as to its requirement by farm animals. As measured by the growth needs of rats, milk is much richer in flavin than in

<sup>1</sup> ANDREWS, VERNON L., Infantile beriberi, *Philippine J. Sci.* **7B**, 67-87, 1912.

<sup>2</sup> GUNDERSON, FRANK L., and H. STEENBOCK, Is the vitamin B content of milk under physiological control? *J. Nutrition*, **5**, 199-212, 1932.

<sup>3</sup> HUSSEMAN, DOROTHY L. and ROSSELENE ARNOLD HETLER, The vitamin B and G requirements of lactation, *J. Nutrition*, **4**, 127-140, 1931.

vitamin B. Donelson and Macy<sup>1</sup> found that, when yeast was fed to lactating mothers, the milk was somewhat enriched in vitamin G (flavin) though not in the antineuritic factor. Failure to increase the flavin content of cow's milk by feeding has been reported.

**336. Vitamin C.**—Since the antiscorbutic vitamin is not required in the rations of farm animals, it has no bearing on lactation except as regards its secretion in milk which is used for human food. Freshly drawn cow's milk always contains ascorbic acid, though not in sufficient amounts to make it a rich source in terms of human needs. The concentration varies among individuals, and there may be breed differences as well; but, contrary to earlier evidence, the feed of the cow has no significant influence. In carefully controlled studies by both biological assays and chemical tests, Riddell and associates<sup>2</sup> found no difference between milk produced on pasture and that produced with dry feeds with or without silage. These findings have been confirmed by other workers. Sharp<sup>3</sup> has shown with the goat that the intravenous injection of ascorbic acid does not increase the potency of the milk.

On standing, the antiscorbutic activity of milk decreases under the influence of destructive factors which are only partially understood at the present time. Under certain conditions, both raw and pasteurized milk may lose all of their potency before they are consumed. Oxidation is concerned in the destruction, and the variations in rate of loss have been explained by the presence of factors accelerating or retarding this oxidation. Assuming the presence of an ascorbic acid oxidase, Sharp considers that the accelerating action of copper is the essential cause of the destruction in commercial pasteurization. He found that, in the absence of copper, milk could be pasteurized by the holder method with a minimum loss and that, if the heating was such as to kill the enzyme, the resulting product had more potency after storage

<sup>1</sup> DONELSON, EVA G., and MACY, G. M., Human milk studies. XII. The vitamin B and vitamin G content before and during maternal consumption of yeast, *J. Nutrition*, **7**, 231-247, 1934.

<sup>2</sup> RIDDELL, W. H., C. H. WHITNAH, J. S. HUGHES, and H. F. LEINHARDT, Influence of the ration on the vitamin C content of milk, *J. Nutrition*, **11**, 47-54, 1936.

<sup>3</sup> SHARP, PAUL F., Vitamin C in pasteurized milk, *Science* **84**: 461-462, 1936.

than the same milk held raw for the same time. Kon and Watson<sup>1</sup> have shown that visible light accelerates the loss of the vitamin. Following exposure to light spontaneous destructive changes occur. A pint bottle of milk exposed under practical conditions on the doorstep for half an hour and then kept for one hour in darkness lost half its original potency. Kon and Watson state that the amount of destruction of vitamin C caused by pasteurization in the absence of catalytic metals depends on the previous exposure of the milk to light. It is clear from several experiments that heating is not the cause of the destruction which commonly occurs in the holder method of pasteurization.

**337. Other Vitamins.**—Reference has been made to the evidence that vitamin E may be required for lactation as well as for reproduction in the rat (Sec. 289). There is no similar evidence for other species. The vitamin occurs in cow's milk.

Recently several investigators have reported that there are lactation factors required by the rat which are different from any of the known vitamins. It is too early to attempt to determine the significance of these reports. Most of the reports mention liver as a source of the factors in question.

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<sup>1</sup> KON, STANISLAW KAZIMIERZ, and MEARN'S BRUCE WATSON, The effect of light on the vitamin C of milk, *Biochem. J.*, **30**, 2273-2290, 1936.

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## CHAPTER XVI

### WORK PRODUCTION

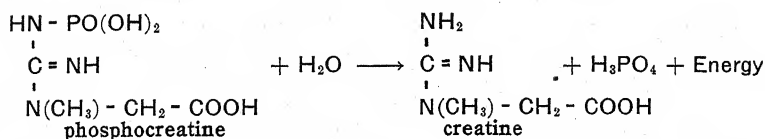
The previous discussions of nutritive requirements have dealt with the production and maintenance of body tissue and with the formation of products composed of nutrients such as milk and eggs. Body maintenance involves the performance of internal work in respiration, circulation, and other vital processes. It also includes a certain amount of external work represented by the voluntary activity of the animal and assessed as the activity factor (Sec. 229) when maintenance is estimated from basal metabolism. The work performed by the horse, and also by the manual laborer, is so greatly in excess of that which properly belongs to maintenance as to call for a special consideration of its physiology and nutritive requirements. The daily energy requirement of the horse or man at hard work is approximately double the maintenance need, while the maximum work which can be performed during a short period may involve a metabolism ten times as great as at rest.

**338. Nutrients Involved in Muscle Activity.**—The muscles are the agencies by which mechanical work is performed. In their contraction, nutrients are catabolized. The early physiologists considered that the muscle was broken down in the process, and, since the muscle was known to consist mostly of protein, the assumption arose that protein yielded the energy for the work done. Such was Liebig's view. For many years he taught that work production involved an increased excretion of nitrogenous end-products and, therefore, required an increased intake of protein in accordance with the amount of work performed. Because of his eminence Liebig's views continued to be accepted, though apparently not based upon experimental results, even after Voit showed in 1860 that work could be performed by a dog without increasing the protein catabolism.

In 1866 Fick and Wislicenus ascended a Swiss mountain 6418 ft. high after having abstained from nitrogenous food for

17 hr. and measured their urea output before and during the ascent. They found no considerable increase in the output while the work was being performed, and their calculations revealed that the total nitrogen excretion could account for only a fraction of the catabolism which must have occurred to furnish the energy needed for the work done. In 1879 Kellner showed that, as long as the total amount of feed of the work horse was ample, the protein catabolism was not increased by work. If, however, the feed was restricted and the work was increased to the point where the animal lost flesh, then a larger nitrogen excretion occurred. Thus it gradually came to be accepted that the muscle is not broken down in work and that its energy is normally supplied by nonnitrogenous food, but that if the food supply is insufficient body protein as well as fat may be drawn on.

**339. The Chemistry of Muscle Action.**—The knowledge of the nature and sequence of the chemical and thermal changes which take place during muscular activity has been undergoing modification during recent years. Many of the details remain unknown or in dispute. It seems to be agreed at present that the following events take place. When a muscle is stimulated, phosphocreatine is hydrolyzed to creatine and phosphoric acid, yielding energy which is partly utilized for the contraction and partly dissipated as heat:



This is the primary reaction which provides the energy for muscle action. But for continued action there must be a renewal of the phosphocreatine. This is accomplished by a resynthesis of this compound during the recovery period. Energy for this process is derived from two further reactions. In one of these glycogen is hydrolyzed to lactic acid, hexose phosphate occurring as an intermediary product. In the second a part of the lactic acid is oxidized to carbon dioxide and water. These two reactions furnish energy not only for the resynthesis of phosphocreatine but also for the reformation of glycogen from the unoxidized lactic acid.

Thus, when the original chemical condition of the resting muscle is restored, the net change which has occurred is the oxidation of lactic acid. It should be noted, however, that this oxidation does not take place until after the contraction occurs. The energy for the contraction is furnished by the anaerobic hydrolysis of phosphocreatine. Thus the process is not the same as occurs in the heat engine where the fuel burns before the work is done. Rather, it is analogous to the operation of a storage battery. Contraction takes place as a result of a discharge of energy stored in the muscle. "Charging" occurs during the recovery period with energy obtained by oxidation. The muscle differs from the heat engine in another fundamental respect. The heat produced by the engine provides the energy for the work accomplished, while in the muscle the energy which appears as heat is entirely a waste. Although it is convenient to evaluate foods and body products in terms of heat units, it should be remembered that the body is in no sense a heat engine.

The fact that an anaerobic reaction furnishes the immediate energy for muscle contraction and that recovery can occur anaerobically by means of the energy supplied by lactic-acid formation makes it possible for muscle action to take place temporarily without oxygen. The process is stopped by the accumulation of lactic acid. Then oxidation is required to remove the acid and to furnish energy for building up the system so it can start again. The anaerobic mechanism also means that the muscle can temporarily do much more work than represented by the amount of oxygen currently required to accomplish it. When this occurs, the deficit of oxygen is spoken of as the *oxygen debt*. This provision of nature enables a man or an animal to exert itself eight or ten times as strenuously as would be possible if all of the oxygen had to be supplied currently. A well-trained athlete cannot take in more than about 4 liters of oxygen per minute, yet he can temporarily perform work which would require oxygen at the rate of 30 liters per minute if it were not possible for him to go into oxygen debt. The same picture can be exhibited by the horse.

**340. Carbohydrate vs. Fat as a Source of Muscular Energy.**—The usual source of muscular energy is carbohydrate, but fat may serve also. Carbohydrate is the more economical fuel in the sense that, per liter of oxygen used, approximately 10 per cent

more energy is liberated from it than from fat. This is of little importance at moderate work but for an intense exertion of short duration, where capacity to take in oxygen becomes the limiting factor, carbohydrate is undoubtedly demanded as the fuel.

The previously described chemical changes of muscle metabolism are worked out on the basis of carbohydrate as the source of the lactic acid oxidized. The question as to whether fat must first be changed into carbohydrate before being burned is still in debate. This view is strongly challenged on the ground that such a transformation has never been demonstrated and particularly that it would involve a loss of energy which actually does not occur when carbohydrate is replaced by fat as the fuel for muscular work.

**341. Units of Work and Power.**—Work done may be measured in *foot-pounds*; and power, the rate of doing work, may be measured in *foot-pounds* per second. The development of 1 *horsepower* (hp.), also a unit of power, necessitates the performance of work at the rate of 33,000 ft.-lb. per minute. The work done in moving a body is measured by the product of the force required and the distance the body moves along the line of action of the force. Suppose that a horse must exert a force of 200 lb. to pull a load at a speed of 3 miles per hour (264 ft. per minute). In pulling the load a distance of 264 ft. the horse does 52,800 ft.-lb. of work. Since the work is done in 1 minute the power developed is simply 52,800 ft.-lb. per minute, and, if this be divided by 33,000, the result is approximately 1.6 hp. One horsepower-hour, also a unit of work, is the energy expended when work is done at the rate of 1 hp. for 1 hr. It is thus 33,000 times 60, or 1,980,000 ft.-lb.

In studying the energy efficiency of work production, it is convenient to translate foot-pounds into Calories. One foot-pound is equal to 0.000324 Cal.

**342. Efficiency of Muscle Work.**—The mechanical efficiency of the animal machine can be computed even as is true for the heat engine. It represents the percentage of the chemical energy used which can be transformed into useful work. The *gross* or *over-all* efficiency is defined by the following equation:

$$\text{Gross efficiency} = \frac{\text{mechanical work accomplished}}{\text{total energy expended while working}}$$

It is obvious that only a part of the total energy consumed is actually used for the production of useful work. A portion must serve for the usual processes of maintenance, another portion is dissipated by the increased internal activities, such as circulation and respiration, which are demanded by muscle action, and a further portion is used up in waste movements. Only 40 to 50 per cent of the energy consumed by the muscle is actually transformed into work. The rest appears as heat in connection with the exothermic chemical processes previously mentioned. Thus the maximum gross efficiency which is shown by the man or horse is approximately 25 per cent. This is a high figure, however, compared to the steam or gas engine, according to the following figures from Hill:<sup>1</sup>

Steam engine without condenser.....	7.5 per cent
Steam engine with condenser.....	9 to 19 per cent
Gas engine.....	14 to 18 per cent

It is a high figure also compared to the tractor which was found to have an efficiency for draft of 13 per cent, in experiments by Brackett<sup>2</sup> and associates.

In comparing the horse and tractor, however, it must be borne in mind that the horse can only work part of the time. His true efficiency for farm labor is what he can accomplish regularly in a 24-hr. day. Brody and Cunningham<sup>3</sup> found the *all-day* efficiency, as measured by metabolism data, of a horse working 8 hr. a day to be 14 per cent. Morrison<sup>4</sup> has arrived at the lower figure of 8.9 per cent, by calculating the amount of work which a 1500-lb. horse could be expected to accomplish daily and the amount of feed which should represent a satisfactory allowance. In comparing the horse with the tractor, Morrison reminds us that the horse uses fuel in a very crude form compared to that of

<sup>1</sup> HILL, A. V., *Muscular Movements in Man*, Cornell University Press, Ithaca, N. Y., 1927.

<sup>2</sup> BRACKETT, E., C. W. SMITH, E. B. LEWIS, CARLTON L. ZINK, and C. F. ADAMS, Nebraska tractor tests, 1930-1934, *Nebr. Agr. Expt. Sta. Bull.* 292, 1935.

<sup>3</sup> BRODY, SAMUEL, and RICHARD CUNNINGHAM, Growth and development. XL. Comparison between efficiency of horse, man, and motor, with special reference to size and monetary economy, *Mo. Agr. Expt. Sta. Research Bull.* 244, 1936.

<sup>4</sup> MORRISON, F. B., Feed and feeding, pp. 428-429, Morrison Publishing Company, Ithaca, N. Y.

the tractor and that it makes its own repairs. The horse far out-classes the tractor in ability temporarily to exceed its normal load because of its capacity for incurring oxygen debt.

Another measure which is used in studies of work production is the *net efficiency*. Here the work accomplished is calculated as a percentage of the total energy intake minus that used for maintenance. Such a figure is a truer measure of the muscular efficiency since it eliminates the overhead expenditure of maintaining the body as a whole. Certain questions arise as to just what should be deducted from the gross expenditure. Should the deduction represent merely the metabolism of the resting animal or should certain movements which are normally made by the idle animal and which do not result in useful work be included in the deduction? Such questions are of minor importance if the same basis is used where efficiencies are compared. Brody defines net efficiency by the following equation:

$$\text{Net efficiency} = \frac{\text{work done}}{\text{total energy used} - \text{energy of standing animal}}$$

As a comparative measure, net efficiency has the advantage over gross efficiency in being less affected by variations in the intensity and amount of work done over a given period.

**343. The Measurement of Energy Expended in Work.**—Zuntz was a pioneer in measuring the energy expended by horses in different kinds of work. Using the respiration apparatus which he devised, he was able to arrive at the energy output by indirect calorimetry. He used a tread power which could be set at various inclinations for measuring the work required for different degrees of ascent. Provision was made for driving the power by a steam engine so that locomotion only could be studied. The energy requirements for carrying various loads were measured. By determining the energy output during rest as well as during work, the amount needed in excess of maintenance and the net efficiency were computed. Many of these experiments are reported by Zuntz and Lehmann.<sup>1</sup>

Brody and associates<sup>2</sup> have recently conducted somewhat similar studies. Some data taken from one of their experiments

<sup>1</sup> ZUNTZ, N., and C. LEHMANN, Untersuchungen über den stoffwechsel des Pferdes bei Ruhe und Arbeit, *Landw. Jahrb.*, 18, 1-156, 1889.

<sup>2</sup> PROCTER, ROBERT C., SAMUEL BRODY, MACK M. JONES, and D. W. CHITTENDEN, Growth and development. XXXIII. Efficiency of work in

are presented in Table XXXIII to illustrate the nature of the results produced. These data were obtained with a Percheron gelding walking at the rate of 2.2 miles per hour. Having measured the draft and the distance traveled in the tread power, the data as to Calories of work accomplished and the horse power

TABLE XXXIII.—ENERGY CONSUMPTION AND OUTPUT BY A WORK HORSE

Experiment	Draft, lb.	Energy output, Cal. per hr.		Energy expense, Cal. per hr.			Efficiency of work, %		
		Work done	Hp.	Over- all	Net	Absol- ute	Over- all	Net	Absol- ute
Standing.....	0	.....	.....	670					
Walking.....	0	.....	.....	1607	937				
Working.....	125	470	0.73	2908	2238	1301	16.2	21.0	36.1
Working.....	150	564	0.88	3113	2443	1506	18.1	23.1	37.5
Working.....	175	658	1.03	3350	2680	1743	19.6	24.6	37.8
Working.....	200	752	1.17	3651	2981	2044	20.6	25.2	36.8
Working.....	225	846	1.32	4031	3361	2424	21.0	25.2	34.9
Working.....	250	941	1.47	4232	3562	2625	22.2	26.4	35.8
Working.....	275	1035	1.61	4650	3980	3043	22.3	26.0	34.0
Working.....	300	1129	1.76	5008	4338	3401	22.5	26.0	33.2
Working.....	325	1223	1.91	5400	4730	3793	22.6	25.9	32.2
Working.....	350	1317	2.05	5651	4981	4044	23.3	26.4	32.6
Working.....	375	1411	2.20	5752	5082	4145	24.5	27.8	34.0
Working.....	400	1505	2.35	6337	5667	4730	23.7	26.6	31.8

were readily computed using the relationships previously defined (Sec. 341). The energy expense was estimated from the oxygen consumed as measured with a closed-circuit respiration apparatus. The total energy consumption was recorded as the over-all energy. The net-energy expenditure is calculated by subtracting the output during standing from the total measured during walking or working. By adding to the energy of standing, the further amount expended in walking, and subtracting this from the over-all consumption, the *absolute energy* used in the work of draft is obtained.

These three different energy expenditures provide the bases for computing the three different measures of efficiency with which

horses of different ages and body weights, *Mo. Agr. Expt. Sta. Research Bull.* 209, 1934. BRODY and CUNNINGHAM, *loc. cit.*



the work recorded is accomplished. The over-all efficiency increases with load because the fixed overhead expenditure of maintenance is thus distributed over an increasing output of useful work. In contrast, the absolute efficiency tends to decrease, reflecting the larger wastage as heat which tends to result from increasing the load on the muscle. The net efficiency represents a balance between the trend to an increased efficiency with load, which results from eliminating the overhead of walking, and the opposite trend in absolute efficiency with increasing load. Thus the figures for net efficiency reveal an increase for the lighter loads only.

**344. Factors Affecting Work Efficiency.**—The previous discussion indicates that various factors influence the efficiency with which work is done and that they affect the various measures of efficiency in different ways. The practical measure is the over-all efficiency because the horse must be maintained whether it is working or not. The net or absolute measure, however, is the more useful for comparing the relative efficiencies with which different kinds of work are accomplished. Increasing the speed beyond a certain point decreases the net efficiency with which work is done. It is evident that a man uses up much more energy in running 100 yd. at top speed than in running it at a trot. Zuntz and associates found that approximately 15 per cent more energy was required by the horse for locomotion at 3.66 miles per hour than at a rate of 2.91 miles. At a trot nearly twice as much energy was expended as at a walk. The horse is most efficient when working at a speed between 2.5 and 3 miles per hour. Though gross efficiency increases with load and speed, a heavy load at low speed is more efficient than a light load at high speed.

Of all the forms of work investigated by Zuntz and associates, the ascent of a moderate grade appeared to be the most efficient on the net basis, but the efficiency decreased as the grade became steeper. Draft up a grade was performed less efficiently than draft alone, and, as the grade increased from 1.5 per cent to 8.5 per cent, the net efficiency decreased from 31.3 to 22.7 per cent.

It is a familiar fact that training increases working efficiency. When the horse or man attempts an unaccustomed task, many unnecessary muscles are brought into play which are not used when skill in performing the work has been acquired.

On the other hand, efficiency decreases as the animal becomes fatigued.

According to the studies of Brody and associates, if large and small horses perform work in proportion to their weights, there is no difference in the gross efficiency with which the work is accomplished. From observations that the maximum over-all efficiency of the 1500-lb. horse, the 600-lb. horse, and the 150-lb. man is approximately 25 per cent in each case, Brody concludes that this efficiency is independent of body weight. He also believes that the work-rate capacity is proportional, not to body weight but to the basal metabolism, *i.e.*, body weight raised to the two-thirds power (Sec. 223).

**345. Energy Requirements.**—The previous discussion of the physiology of muscular work makes it evident that the major requirement is for energy-producing food. This need is most easily visualized as net energy, representing the chemical energy which the body must expend to produce the work in question. This net-energy requirement can be directly measured by respiration experiments such as the one reported in Table XXXIII. As an alternative procedure, the net-energy requirement can be calculated from the energy represented by the work done and by the percentage efficiency represented by the work in question. This procedure is illustrated by Armsby<sup>1</sup> as follows. He cites data showing that a horse, hauling a load having a draft of 100 lb. for 20 miles on a level road, would perform mechanical work equivalent to 3421 Cal. Taking the net efficiency of the horse for draft as 31.3 per cent, the calculation thus becomes:

$$3421 \div 0.313 = 10,929 \text{ Cal. of net energy}$$

This calculation accounts only for the energy needed for the work of draft itself. The horse expended energy for walking and for maintenance which must be added to that of the draft accomplished in arriving at the total net-energy requirement.

A little thought makes it evident that calculations of this kind have a very limited application to the estimation of the net-energy requirements of horses in practice because of the difficulty of arriving at the amount of work done and the efficiency with

<sup>1</sup> ARMSBY, HENRY PRENTISS, *The nutrition of farm animals*, p. 564, The Macmillan Company, New York, 1917.

which it is performed. Armsby recognized this fact and, in stating the requirements for work production, he limited them to the basis of "full work," "half work," and "one-fourth work." For example, his recommendation to cover both the maintenance and production of a horse at full work was 18.2 Therms of net energy per day per 1000 lb. live weight. Armsby recognized that there were no directly determined net-energy values of feeds for work production by the horse. This fact, coupled with the more recent recognition of the variability of such values according to the level of production and the nature of the ration (Sec. 218), has prevented the adoption of the net-energy system in computing rations for horses.

Brody and Cunningham<sup>1</sup> have derived the following equation for expressing the over-all requirements of the horse in terms of pounds of total digestible nutrients:

$$\text{T.D.N.} = 0.053M^{0.73} + 1.27 \text{ (hp.-hr.)}$$

The first term to the right of the equals sign represents the maintenance requirement in Calories when  $M$  is live weight in pounds. The work requirement is obtained by multiplying horsepower-hours by the factor 1.27, to give the Calories representing the work done. One pound of T.D.N. is considered equivalent to 1814 Cal. Brody and Cunningham present an alignment chart or nomograph from which the requirements for animals of different weights performing varying amounts of work can be obtained at a glance. Their computed values for different body weights are also set forth in tables in terms both of the horsepower-hours of work done and also of the number of hours worked per day. As an example, the requirement for a 1000-lb. horse working an 8-hr. day is stated as 14.1 lb. of total digestible nutrients. Their values are based on an assumed tractive pull of a load equivalent to 10 per cent of the body weight and upon a speed of 2.2 miles per hour.

The energy requirements for the horse can be determined in feeding trials by ascertaining the amount of feed which is needed to maintain the animal in weight and in good working condition while doing a definite amount of work. The feed intake can be expressed as total digestible nutrients, either by running a digestion trial or by using coefficients of digestibility which have been

<sup>1</sup> BRODY and CUNNINGHAM, *loc. cit.*

previously determined for the feeds in question. In translating the results into a feeding standard, a difficult problem arises in estimating the amount of work actually performed on a basis which will apply to other conditions. In addition to the factors previously mentioned (Sec. 344) as influencing work efficiency, there are differences in working conditions from day to day which markedly affect the amount of feed required to do a given amount of work. The nature of the roadbed, whether hard, soggy, or icy, is an important example in this connection. Feeding standards are useful to indicate how the feed requirements vary in accordance with amount of work performed, but, in practice, they can serve only as general guides. Having selected a suitable ration, it should be fed in accordance with the amounts needed to keep the horse in good working condition rather than as arbitrarily specified allowances.

**346. Protein Requirements.**—While it is now accepted, as discussed at the beginning of the chapter, that protein is not the normal fuel of muscular work, some still adhere to the view that protein catabolism is increased during the work even though there is an ample supply of nonnitrogenous nutrients. To many, it is inconceivable that the muscle cells are entirely resistant to wear, and they believe that destruction and renewal must occur. It is stated that such a destruction may occur and yet not be reflected in an increased excretion because of a reutilization of the catabolic products. Such a viewpoint is very difficult to prove or disprove. There are experiments in which an increased output of urinary nitrogen has been recorded during work and others in which no such increase has been found. At least some of the positive experiments are inconclusive because of the uncertainty as to whether the intake of nonnitrogenous nutrients was adequate to meet the needs for energy.

Cathcart,<sup>1</sup> from a review of various experiments of others in conjunction with his own, believes the conclusion to be unmistakable that "muscle activity does increase, if often only in a small degree, the metabolism of protein." From a survey of the evidence Lusk<sup>2</sup> comes to the opposite conclusion in equally positive

<sup>1</sup> CATHCART, E. P., Influence of muscle work on protein metabolism, *Physiol. Rev.*, 5, 225-242, 1925.

<sup>2</sup> LUSK, GRAHAM, The science of nutrition, 4th. ed., Chap. XV, W. B. Saunders Company, Philadelphia, 1928.

terms. These two divergent viewpoints persist although there have been later experiments contributing to the problem. Both agree that there is no basis for believing protein to be the source of muscular energy. Lusk states that large intakes of protein are not essential for the maintenance of physical power, and that, on the other hand, there is no proof of a decreased physical efficiency from large intakes.

From the standpoint of an efficient ration for work production, other considerations appear more important than the question as to whether the protein requirement is actually increased during work. A satisfactory maintenance ration for an idle horse has a nutritive ratio of approximately 1:10. During hard work the requirement for energy is approximately doubled, and the nutritive ratio is doubled accordingly if the protein intake is not increased. Working horses have been kept in satisfactory condition on even wider ratios than would here result but that such rations are equally efficient in terms of energy utilization has not been shown. Reference has been made to the fact that digestibility is depressed by wide ratios and that, at least in so far as rats and pigs are concerned, such a ration increases the heat losses (Sec. 218). While these questions have not been specifically studied with the horse, indirect evidence suggests that its protein intake should be increased during work although the increase is not specifically needed for muscular activity. Using feeds of a quality which experiments and practice have shown to be desirable in the ration of the work horse, there is no problem in holding the nutritive ratio substantially the same as recommended for maintenance. It is believed that this practice should be followed.

**347. Mineral Requirements during Work.**—The large increase in the output of sodium and chlorine in the perspiration incident to hard work, particularly during warm weather, has been mentioned (Sec. 120). Thus there is an increased need for salt by the working horse. This need can be taken care of by *ad libitum* feeding and requires no special attention.

The active phosphorus metabolism which occurs during muscular activity (Sec. 339) has directed attention to the question of an increased requirement for this mineral. It is possible that some of the liberated phosphoric acid escapes into the fluids instead of being reunited with creatine and that it may be in turn excreted.

The evidence on this point is contradictory. There is, however, some rather convincing evidence that the ingestion of phosphates enhances the ability to do work, or stimulates the inclination to work. Further study is needed to establish the importance of these observations.

Some increase in hemoglobin destruction and resynthesis during hard work is to be expected in view of the greatly increased activity in oxygen transport, but this does not necessarily involve a higher iron requirement because the catabolized iron can be used again. Studies by Caine<sup>1</sup> revealed no benefit, either in condition or in feed economy, from adding supplements of calcium, phosphorus, and iron to a ration of timothy hay, corn, oats, and salt. The added feed which must be given to supply the energy for work production inevitably means a mineral addition also. If the ration contains the various essential minerals at percentage levels which are adequate for maintenance, it should certainly prove satisfactory in this respect for work production. Brood mares represent a special case calling for attention in accordance with the increased needs for gestation and lactation.

**348. Vitamin Requirements.**—There is no basis at the present time for making any statements as to whether muscular activity calls for an increased intake of any of the vitamins. The recent evidence that vitamin B may be concerned in carbohydrate metabolism is suggestive only. Practically nothing is known regarding the vitamin requirements of the horse for any purpose.

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